Vitamin B_{12}



MERCK SERVICE BULLETIN

MERCK & CO., INC
Chemical Division
RAHWAY NEW JERSEY

Copyright 1958 by Merck & Co Inc Rahway N J
Printed in U S A

Foreword

This *Service Bulletin*, another in the Merck series presents comprehensive, up-to-date information on vitamin B_{12}. Its primary purpose is to aid the pharmaceutical manufacturer, and it is hoped that much of the material will prove equally helpful to others who are interested in the subject. Information essential to the development of new product forms and to the improvement of existing ones is included, as well as basic facts on the nature, physiologic role and clinical uses of the vitamin.

Hence in this volume on Vitamin B_{12} you will find—

Chemical, Pharmaceutical, and Analytical Information

including data on the chemistry and physical properties of vitamin B_{12}

pharmaceutical applications, with technical details concerning injectable solutions containing vitamin B_{12}, tablets of vitamin B_{12} alone or with other B vitamins, and multivitamins, capsules, oral liquids, plus a discussion of compatibilities

assay methods chemical, biological, and microbiological

Nutritional, Metabolic, Physiologic, Pharmacologic, and Clinical Information

comprehensive discussions of the roles of vitamin B_{12} in human nutrition and metabolism are presented, together with data on absorption and excretion

clinical uses in anemias, neurologic disorders, dermatoses, and other conditions are detailed, together with clinical indications for dietary supplementation

Selected Annotated Bibliography—of more than 300 abstracts of selected material appearing in the medicinal and related scientific literature since July 1953

To assist manufacturers still further Merck Technical Service is always ready with even more recent information gained by Merck vitamin research pharmaceutical experience and analyses of current publications—for the solution of your specific problems

CONTENTS

Part 1 Chemical Pharmaceutical and Analytical Information

t 2 Nutritional, Metabolic, Physiologic, Pharmacologic and Clinical Information

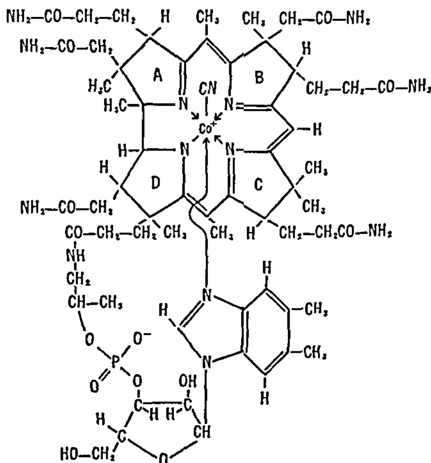
Part 3 Selected Annotated Bil

Part 1

Chemical, Pharmaceutical and Analytical Information

Vitamin B_{1} , also known as cyanocobalamin, is a complex coordination compound containing trivalent cobalt with a coordination number of six Cyanocobalamin has a net charge of zero The cyano group contributes one negative charge and satisfies one coordinate position The cobalt is coordinated to one other negatively charged group and to four groups that carry no local charge All of these coordinating atoms are nitrogen and all five of these groups are linked together into a single moiety The remaining positive charge in the cobalt coordination sphere is balanced by the negatively charged phosphate portion of the molecule

Extensive structural and degradation studies of cyanocobalamin have shown it to have an empirical composition corresponding to the formula $C_{63}H_{88}N_{14}O_{14}P$ Co and a molecular weight of 1355 By a combination of chemical and X-ray crystallographic studies the molec



ular structure has been established, with reasonable certainty, and the formula on the preceding page has been suggested[8]

Vitamin B_{12} has been specifically designated cyanocobalamin, the name cobalamin thus referring to all of the molecule except the cyano group A new system of nomenclature for the analogs and degradation products of vitamin B_{12} has been proposed[9] The fundamental ring system consisting of the four pyrrole nuclei joined in a macro ring with the three bridge carbon atoms and six conjugated double bonds, but without cobalt or side chains, is called "corrin" Cyanocobalamin and its degradation products can be named as corrin derivatives but for convenience other names have been assigned For example the cobalt-containing heptacarboxylic acid resulting from hydrolysis of all of the amide groups present in vitamin B_{12}, without the cyano group and the nucleotide, is designated "corphyrinic acid" The corresponding hexacarboxylic acid which still retains the 2-hydroxy propionamide group on the appropriate side chain is called "corphinic acid" and the hexacarboxylic acid having the ribofuranosidophosphorylpropionamide side chain is called "cobamic acid" Thus cobamide, the hexamide of cobamic acid, would be derived from vitamin B_{12} by removal of the cyano and the 5 6-dimethylbenzimidazole groups By this proposed system of nomenclature cyanocobalamin may be called "cyano 5,6-dimethylbenzimidazolyl cobamide" or "5,6-dimethylbenzimidazole cyanocobamide"

Cyanocobalamin as a whole, is a neutral molecule The cyano group attached to the cobalt atom can be replaced by other ions or groups with strong coordinating tendencies to yield other cobalamins[10] such as hydroxocobalamin chlorocobalamin nitrocobalamin, and thiocyanatocobalamin Hydroxocobalamin combines with hydrogen ion to form positively charged aquocobalamin which can form salts with anions less strongly coordinated than water All these cobalamins are readily converted to cyanocobalamin by treatment with cyanide ion In alkaline solutions containing an excess of cyanide ion cyanocobalamin takes up a second cyanide group to form dicyanocobalamin the so-called "purple complex," which is unstable and exists only in solution in an excess of the cyanide ion

In glacial acetic acid solution, vitamin B_{12} reacts with perchloric acid to form an orange colored perchlorate The compound is of structural interest insofar as its formation confirms the presence of at least six weakly basic groups in the vitamin molecule[11]

ture The anhydrous compound, however, is very hygroscopic, and when exposed to moist air may absorb about 12 per cent of water Although vitamin B_1 is slowly decomposed by ultraviolet or visible light, the crystalline solid and its aqueous solutions show no significant decomposition during exposure at room temperature to normal indoor illumination or indirect sunlight for the time normally required for pharmaceutical processing Prolonged exposure to strong light, however, should be avoided

Crystalline vitamin B_{12} is stable at normal temperatures Aqueous solutions at *p*H 4 0 to 7 0 show no decomposition during extended storage at 25°C For optimum stability at elevated temperatures, solutions should be adjusted to *p*H 4 0 to 4 5 Aqueous solutions in this *p*H range may be autoclaved for 20 minutes at 120°C without significant decomposition

Cobalamin Concentrate N F (Vitamin B_{12} Activity Concentrate) is a partially purified product, resulting from the growth of a cobalamin producing microorganism, which contains harmless diluents or stabilizing agents It occurs as pink to brown powder or granules which may be hygroscopic The chemical and physical properties vary depending upon the specific composition The potency is stated in terms of the number of micrograms of cobalamin in each gram of cobalamin concentrate The cobalamin content is determined by the Cobalamin Assay described in the Second Interim Revision Announcement, *National Formulary X* [25] *The identity tests and other require*ments for Cobalamin Concentrate N F are described on page 167 of The National Formulary, Tenth Edition

Cobalamin Concentrates (Vitamin B_{12} Solids) N F, adjusted to a uniform potency of either 500 or 1,000 mg of cobalamin (vitamin B_{12} activity) per Kg with anhydrous dibasic calcium phosphate, are supplied, for pharmaceutical manufacturing in screw capped bottles or fiber drums containing from 100 mg to 10 Gm of cobalamin

Cobalamin Concentrates (Vitamin B_{12} *Solids) N F*, *adjusted to a* uniform potency of either 1,000 mg or 3,000 mg of cobalamin (vitamin B_{12} activity) per Kg with a water soluble carrier, mannitol, are supplied, for pharmaceutical manufacturing, in screw-capped bottles or fiber drums containing from 100 mg to 10 Gm of cobalamin

A sterile aqueous solution of Cobalamin Concentrate N F, adjusted to a minimum potency of 5,000 micrograms of vitamin B_{12} activity per cc, is supplied, for manufacture of parenteral solutions,

in 200, 1,000 and 5,000 cc bottles containing 1, 5 and 25 Gm, respectively, of cobalamin. This solution also contains 1.5 per cent of benzyl alcohol as a preservative.

In all of the Cobalamin Concentrates N.F. supplied by Merck, the labeled cobalamin content is present entirely as cyanocobalamin in noncrystalline form.

PHARMACEUTICAL APPLICATIONS

Some of the pharmaceutical properties of crystalline vitamin B_{12} were described soon after it became available for therapeutic and nutritional use.[25-27] Experience in the manufacture of a wide variety of pharmaceutical products, as well as critical studies employing accelerated stability tests and long term storage tests, have shown that many stable products containing crystalline vitamin B_{12} may be prepared by conventional manufacturing procedures. Crystalline vitamin B_{12} has been shown to be compatible with a wide variety of therapeutic and nutritional substances.[28] Those few substances which are chemically incompatible with vitamin B_{12} in aqueous solutions may be incorporated into many dry formulations by special procedures which minimize their adverse effect on the stability of the vitamin.

The procedures to be described for the preparation of various dosage forms of vitamin B_{12} have been used in the experimental production of pharmaceutical formulations. Manufacturers adapting these methods to their own operations should first prepare trial lots in order that a finished product meeting their specific requirements may be assured.

Injectable Solutions Containing Vitamin B_{12}

Aqueous solutions of vitamin B_{12} for parenteral administration are usually prepared in a saline vehicle containing a suitable preservative. Vitamin B_{12} in solution is sensitive to ultraviolet or visible light and during all stages of processing should be protected from direct sunlight and bright artificial light. Exposure to bright light should also be minimized during subdividing, inspection and packaging. Since vitamin B_{12} is a growth stimulant for many microorganisms, the solutions should be sterilized as soon as possible.

Only glass, glass lined, or enameled equipment should be used in processing the saline solutions. Corrosion of stainless steel equipment by saline solutions may introduce metallic contaminants. Stainless

steel needles have been found suitable for use in filling equipment, however, because of the relatively short period of contact A surgical grade of rubber tubing should be used, if needed, for connections in the filling equipment

Crystalline vitamin B_{12} (Cyanocobalamin U S P) or a suitable solution of cobalamin concentrate may be used for the manufacture of parenteral solutions

Single-Dose All-Glass Ampuls of Vitamin B_{12}

Solutions for subdivision into single dose ampuls may be prepared with water for injection or with an isotonic solution of sodium chloride in pyrogen-free distilled water The *p*H of the vehicle should be adjusted to between 4 0 and 4 5 before the vitamin B_{12} is added The required amount of crystalline vitamin B_{12}, calculated on the anhydrous basis (including 5 per cent overage), is dissolved in the aqueous vehicle To prevent bacterial growth and subsequent loss of potency during subdivision, the solution is immediately sterilized by filtration through a microporous porcelain candle The candle should be autoclaved and aseptically rinsed with water before use The solution is collected in sterile bulk containers and aseptically subdivided into previously washed and sterilized ampuls of Type I glass The ampuls are sealed and immediately sterilized by autoclaving for 20 minutes at 120°C

Multiple-Dose Vials of Vitamin B_{12}

Injectable solutions containing crystalline vitamin B_{12} may be prepared with water for injection or with an isotonic solution of sodium chloride in pyrogen free distilled water The vehicle should contain a suitable bacteriostatic agent and the *p*H should be adjusted to 4 0 to 4 5 before the vitamin B_{12} is added The vehicle may be clarified, if necessary, by filtering through a fritted glass filter of medium porosity The desired weight of crystalline vitamin B_{12}, calculated on the anhydrous basis (including 5 per cent overage), is dissolved in the aqueous vehicle

The solution is immediately sterilized by filtration through a microporous porcelain candle which has been autoclaved and then aseptically rinsed with water Heat sterilization of the solution is not recommended when a bacteriostatic agent is used The filtered solution is collected in suitable sterile bulk containers, and then subdivided into

previously washed and sterilized vials of Type I glass The vials are immediately sealed by affixing sterile stoppers which have been previously tested for compatability with vitamin B_{12} solutions

The stoppers are washed in a suitable detergent solution, rinsed with distilled water until free of detergent, then immersed in distilled water and autoclaved at 120°C for 20 minutes After the water is poured off, the stoppers are rinsed with distilled water Sufficient distilled water is added to cover the stoppers which are again autoclaved at 120°C for 20 minutes After rinsing with distilled water, the stoppers are dried and sterilized

Vitamin B_{12} with Other B Vitamins

Injectable solutions containing crystalline vitamin B_{12} and other B vitamins may be prepared using an isotonic solution of sodium chloride in pyrogen free distilled water as the vehicle

About 90 per cent of the required amount of isotonic sodium chloride solution is heated to 95°C The niacinamide (including 5 per cent overage) is dissolved in the saline solution, and riboflavin (includ ing 5 per cent overage) is added and stirred until solution is complete The solution is then allowed to cool to room temperature

Thiamine hydrochloride, ampul grade (including 20 per cent overage) pyridoxine hydrochloride (including 10 per cent overage) and calcium pantothenate dextrorotatory (including 50 per cent overage) are dissolved in the cooled solution The solution is immediately adjusted to pH 4 0 to 4 5 and a suitable preservative is added

The required amount of crystalline vitamin B_1 , calculated on the anhydrous basis, is dissolved in the solution and sufficient quantity of the isotonic sodium chloride vehicle added to make the desired total volume An overage of 10 per cent of crystalline vitamin B_{12} has been found adequate in solutions containing up to 10 mg per cc of thiamine hydrochloride When higher concentrations of thiamine are employed, adequate testing must be done to determine the necessary overage

The solution is sterilized by filtration, using a microporous porcelain candle which has been autoclaved and then aseptically rinsed with water

The sterile solution is collected in suitable sterile bulk containers It is then subdivided under aseptic conditions either into previously washed and sterilized glass ampuls which are immediately flame

sealed, or into previously washed and sterilized vials which are imme diately sealed with sterile stoppers Stoppers of a quality suitable for use with such solutions should be washed and prepared as described above

Vitamin B_{12} and Other B Vitamins with Liver

Injectable solutions containing crystalline vitamin B_{12} and other B vitamins with liver may be prepared by the method described for injectable solutions containing vitamin B_{12} with other B vitamins The required amount of Liver Injection Crude U S P is added before the *p*H of the solution containing the other B vitamins is adjusted The solution is then adjusted to *p*H 4 0 to 4 5 and a suitable bacteriostatic agent added The required amount of crystalline vitamin B_{12}, calcu lated on the anhydrous basis (including 10 per cent overage), is dis solved in this solution and sufficient quantity of the isotonic sodium chloride vehicle added to make the desired total volume

The solution is sterilized by filtration, using a microporous porce lain candle which has been autoclaved and then aseptically rinsed with water The sterile solution is collected in suitable sterile bulk con tainers, and stored at about 5°C for one week to permit coalescence of any insoluble material

If necessary, the solution is again aseptically filtered as described above and the clear solution is subdivided under aseptic conditions into previously washed and sterilized vials

TABLETS

Oral tablets containing vitamin B_{12} alone or in combination with the other B vitamins may be prepared by conventional procedures as described

Tablets of Vitamin B_{12}

Tablets containing vitamin B_{12} may be prepared by a wet granula tion procedure using as the diluent a powdered mixture of 7 parts of mannitol and 1 part of dibasic calcium phosphate

The required amounts of a suitable trituration containing crystal line vitamin B_{12} or cobalamin concentrate (including 10 per cent over age) and the diluent are thoroughly mixed and sifted through a 40-mesh stainless steel screen The powder is moistened with a freshly prepared 10 per cent cornstarch paste The wet mass is forced through

an 8 mesh stainless steel screen and dried at 40 C The dried granules are forced through a 14 mesh stainless steel screen and then lubricated with a mixture of magnesium stearate and cornstarch The granules are then compressed into tablets

Soluble, flavored tablets of crystalline vitamin B_{12} which are easily crushed, yet hard enough to withstand careful handling, may be manufactured by the following method A lactose granule is prepared by moistening 94 parts of lactose with distilled water containing a suitable red dye The damp mass is passed through a 12 mesh stainless steel screen and dried first at room temperature for approximately 16 hours, then at 40°C for 1 hour The dried granules are forced through a 16-mesh screen and the fines removed through a 40-mesh screen The required amount of 0 1 per cent Trituration of Crystalline Vitamin B_1 with Mannitol is mixed with sufficient mannitol to provide 5 parts of the total weight of the compression mixture Suitable quantities of sodium saccharin and flavor are added and the mixture is thoroughly blended with the fines from the lactose granule This powdered mixture and 1 part of magnesium stearate are added to the dried lactose granules and, after thorough mixing, compressed into tablets of suitable hardness

Tablets of Vitamin B_{12} with Other B Vitamins

Oral tablets of the B vitamins with vitamin B_{12} may be prepared by mixing the required amounts of a prepared trituration containing crystalline vitamin B_{12} or cobalamin concentrate, riboflavin pyridoxine hydrochloride and niacinamide, including a 10 per cent overage of each vitamin A diluent, consisting of a mixture of 4 parts of mannitol and 1 part of cornstarch, is added during continuous blending The weight of the diluent should be at least five times that of the vitamin mixture

After sifting the mixture through a 40 mesh stainless steel screen, the powder is moistened with a warm 10 per cent solution of gelatin and the wet mass pressed through an 8 mesh stainless steel screen The resulting granules are dried at 40°C

The dried granules are forced through a 14 mesh stainless steel screen The fines are removed through a 40 mesh stainless steel screen and divided into three portions The required amount of thiamine mononitrate (including 10 per cent overage) is mixed with one portion of the fines and sifted onto the granules through the 40 mesh screen

The required amount of calcium pantothenate dextrorotatory (including 50 per cent overage) is mixed with a second portion of the fines and sifted onto the granules through the 40-mesh screen The third portion of fines is thoroughly mixed with suitable lubricants and sifted onto the granules through a 40 mesh screen The lubricated granules are then mixed and compressed into tablets

Tablets of Vitamin B_{12} and Intrinsic Factor

Tablets containing vitamin B_{12} and intrinsic factor concentrate may be prepared by a "slugging" (dry compression) procedure The required amount of Vitamin B_{12} with Intrinsic Factor Concentrate U S P is mixed with suitable diluents such as lactose or dicalcium phosphate and a lubricant such as magnesium stearate The mixture is compressed into slugs which are reduced to a 12 mesh granule, and then recompressed into tablets of the desired size

Multivitamin Tablets

Oral multivitamin tablets, such as the Decavitamin Tablet described in U S P XV, usually include vitamins A and D, ascorbic acid, *d* calcium pantothenate, crystalline vitamin B_{1} , folic acid, niacinamide, pyridoxine hydrochloride, riboflavin and thiamine hydrochloride or mononitrate The quantities of the components may be varied to prepare tablets containing the desired maintenance or therapeutic levels

In the preparation of such tablets the required amount of a suitable trituration containing crystalline vitamin B_{12} is mixed with the required amounts of riboflavin, niacinamide and pyridoxine hydrochloride, including 10 per cent overage of each vitamin A separate granulation of this mixture is prepared by conventional procedures using suitable granulating solutions such as 2 per cent ethyl cellulose in 95 per cent alcohol or 10 per cent zein in warm 95 per cent alcohol

The required amounts of granular vitamins A and D (including 10 per cent overage), powdered thiamine mononitrate (including 10 per cent overage), powdered *d* calcium pantothenate (including 50 per cent overage), and ascorbic acid, fine or medium crystals (including 10 per cent overage), are added to the dried granulation together with suitable lubricants and thoroughly blended The mixture is then compressed into tablets and a sugar coating applied by conventional methods

may be advisable in dry filled capsules containing reactive mixtures which are influenced by moisture

The incorporation of vitamin B_{12} alone or in combination with vitamin mixtures and other substances with which it is compatible into hard gelatin capsules is accomplished by mixing the components with suitable dry extenders such as cornstarch, mannitol, or dicalcium phosphate The methods used in preparing uniform mixtures of dry powders for tablets are applicable to capsule manufacture A small amount of magnesium stearate may be added as a lubricant

Methods for incorporating vitamin B_{12} and other vitamins into soft gelatin capsules have been developed by manufacturers possessing the highly specialized equipment required for this product form The desired combination of vitamins is usually mixed with a suitable base to form a smooth paste which is then encapsulated in soft gelatin on automatic or semiautomatic machines

Oral Liquids

Oral liquid vitamin formulations are usually prepared in vehicles containing sucrose, sorbitol, glycerin, propylene glycol, or combinations of these, with varying amounts of water Such vehicles with low water content have been found suitable for use in liquid multivitamin preparations containing crystalline vitamin B_{12}

A typical oral liquid formulation containing vitamin B_{12} with other B vitamins and hematinic substances may be prepared in a vehicle containing 90 parts (weight/volume) of an invert sugar concentrate consisting of 60 per cent invert sugar and 40 per cent sucrose

The required amounts of ferrous sulfate and Liver Extract U S P and sufficient citric acid to adjust the final product to *p*H 3 5 to 4 0 are dissolved, with stirring, in half of the volume of distilled water required to provide a final volume of 100 parts The solution is kept at room temperature for 48 hours and then clarified by filtration through a suitable cloth filter

The required amounts of niacinamide, riboflavin, and a suitable preservative are dissolved, with the aid of heat, in the total quantity of invert sugar concentrate This solution is cooled to about 35°C, and the aqueous liver and iron solution is then added

The required amounts of thiamine hydrochloride, pyridoxine hydrochloride, choline chloride, and crystalline vitamin B_{12} are dissolved in the remainder of the water Suitable flavoring and coloring

agents are added and this solution is mixed with the invert sugar solution containing the liver and iron

The final product is clarified by filtration and subdivided into bottles

Compatibility in Pharmaceutical Products

Cyanocobalamin is more stable than any of the other cobalamins in pharmaceutical formulations even in the presence of reducing substances

In all types of dry pharmaceutical formulations crystalline vitamin B_{12} is usually stable for prolonged periods The purity of the other components, however, becomes important because the concentrations of vitamin B_{12} employed are usually relatively minute In liquid formulations, trace contaminants, the concentration of the individual components and storage conditions have a marked influence on the stability of vitamin B_{12} Only a few substances are known to have an adverse effect on vitamin B_{12} stability Ascorbic acid causes the most serious difficulty particularly in liquid formulations Other reducing substances such as ferrous salts and aldehydes may also cause decomposition of vitamin B_{12} in some types of pharmaceutical products Specially prepared powdered products containing crystalline vitamin B_{12} in gelatin have been used successfully in dry formulations containing reducing substances such as ascorbic acid and ferrous sulfate

The most important stability problems in pharmaceutical preparations are concerned with the combination of vitamin B_{12} with ascorbic acid in liquid oral products and its combination with thiamine hydrochloride and niacinamide in B complex parenteral solutions Cyanocobalamin, however was found to be the most stable of a series of cobalamin analogs tested in ascorbic acid solutions in 1 molar acetate buffer at *p*H 4 0 Vitamin B_{12} activity concentrates in which all of the activity is present as cyanocobalamin are more stable in such solutions than concentrates containing other cobalamin analogs [16]

The adverse effect of ascorbic acid on vitamin B_{1} stability in oral liquid multivitamin preparations can be reduced by use of vehicles of low water content Sorbitol, propylene glycol or glycerin solutions containing varying amounts of water have been used Vitamin B_{12} in combination with ascorbic acid in a vehicle composed of equal parts of propylene glycol and glycerin has been reported to be stable at room temperature for prolonged periods [29]

The stability of vitamin B_1 in solutions of other B vitamins may vary, depending upon the concentration of thiamine hydrochloride and niacinamide present, the storage temperatures, and the *p*H of the solution At room temperature, vitamin B_1 is stable for prolonged periods in solutions containing as much as 10 mg per cc of thiamine hydrochloride and an equal quantity of niacinamide At levels of 25 to 100 mg per cc of thiamine hydrochloride and niacinamide, vitamin B_1, may decompose during normal storage periods even at room temperature [30] Since the rate of decomposition of vitamin B_{12} in such combinations appears to be directly related to thiamine decomposition, parenteral solutions should be formulated to provide optimum thiamine stability The presence of even small amounts of thiamine decomposition products, or more specifically, those derived from the thiazole moiety, causes rapid decomposition of vitamin B_{12} at elevated temperatures [31] This effect is more pronounced in the presence of niacinamide, although niacinamide itself has been shown to have no adverse effect on vitamin B_{12} stability in solutions held at 120°C for 2 hours [27]

Under normal storage conditions, no serious stability problems have been encountered with vitamin B_{12} in combination with the common sugars in dry mixtures, dilute solutions or syrups In test solutions heated to 120°C , however, significant decomposition of vitamin B_{12} has occurred in solutions containing sucrose Decomposition to a lesser degree has occurred in similar solutions containing dextrose and lactose, but solutions of vitamin B_{12} containing mannitol showed no decomposition after heating to 120°C for several hours

Although talc cannot be considered incompatible with vitamin B_{12}, assay difficulties may result from its use as a filter aid or tablet component because of its ability to adsorb the vitamin

A rapid method for determining chemical compatibility in aqueous solution consists of heating a buffered solution of crystalline vitamin B_1 and the material to be tested at 100°C for 4 hours If the assays of this solution and a control solution containing only the buffer and the vitamin B_{12} differ by less than 10 per cent, the substance under test can be presumed to be compatible with vitamin B_{12} A 1 *M* sodium acetate —acetic acid buffer at *p*H 4 has been found satisfactory for this test Decomposition of vitamin B_{12} in the test solutions at 100°C does not necessarily imply that decomposition will occur in the finished product under normal storage conditions

Biologically, vitamin B_{12} is one of the most potent substances ever discovered Before vitamin B_{12} was discovered and characterized as a vitamin the activity of liver extract was expressed in anti pernicious anemia (APA) units With the introduction of microbiological assay methods, the LLD unit was defined in terms of increased growth produced by vitamin B_{12} in cultures of *Lactobacillus lactis Dorner* After pure crystalline vitamin B_{12} cyanocobalamin was shown to have these two activities (APA and LLD), the weight of vitamin B_{12} equivalent to each of these units was carefully established One microgram of crystalline vitamin B_{12} is equivalent to one APA unit or to eleven thousand LLD units Doses and concentrations of vitamin B_{12} are now expressed in terms of weight of cyanocobalamin Because of the extremely low levels which can be detected, the unit of weight may be the microgram (0 000001 Gm) the millimicrogram (0 000000001 Gm) or even the micromicrogram (0 000000000001 Gm)

The method of choice for the assay of vitamin B_{12} depends upon the purpose of the determination and the nature of the material to be analyzed Each type of assay offers certain advantages and disadvantages Of the microbiologic methods the *Ochromonas* assay is best suited for determination of true vitamin B_{12} activity The microbial assays, however, are more practical for routine purposes when the presence of pseudovitamin B_{12} is not a complicating factor

Many substances have been discovered which resemble the cobalamins in some properties but which lack vitamin B_{12} activity in man and animals At present, the most reliable methods for the determination of vitamin B_{12} activity are the radioisotope tracer assay and the biologic assays based on animal growth When identity as a cobalamin is assured, however, many alternative methods can be selected, based on precision speed, and concentration of sample

Chemical Methods

Most chemical methods for the analysis of vitamin B_{12} can be used successfully only on samples which are moderately concentrated and free of interfering substances

Spectrophotometric Assay

Cyanocobalamin may be identified and assayed by its light absorption spectrum in the visible and ultraviolet regions The United States

Pharmacopeia, Fifteenth Revision, specifies a spectrophotometric method in which the assay may be calculated from the absorbance at 361 millimicrons if the absorbance maxima and their ratios are within the specified limits [3] The absorptivity of cyanocobalamin at 361 millimicrons is 207 The U S P test entitled "Pseudo cyanocobalamin" determines the presence of any similarly absorbing substances which are extractable from cresol carbon tetrachloride with 25 per cent sul furic acid This extraction procedure provides, in some measure, the specificity inherent in the extraction methods of the radioisotope tracer assay discussed below

In the absence of interfering substances, the spectrophotometric method can be used for assaying triturations, solutions and other simple pharmaceutical formulations to which cyanocobalamin has been added The direct spectrophotometric method is speedy and accurate for such assays and may be used to determine as little as 50 micrograms of cyanocobalamin Its usefulness is limited, however, in the assay of products containing vitamin B_1 like compounds or other colored interfering substances

Countercurrent Distribution Assay

The distribution coefficient of cyanocobalamin between mutually saturated water and benzyl alcohol is 1 2 A multiple extraction sys tem of identification and assay based on this property has been described [33] The method is especially suited to the determination of the cyanocobalamin content of crystalline vitamin B_1, and similar products of relatively high purity

Radioisotope Tracer Assay

A combination of selective extractions and adsorptions has been described which purifies cyanocobalamin solutions sufficiently for spectrophotometric determination [34] The fraction of the original cyano cobalamin recovered in the final extract is determined by use of tracer cyanocobalamin containing radioactive Co^{60}, thus permitting calcula tion of the amount of cyanocobalamin in the unpurified sample

The cobalamin assay, described in the First Supplement to U S P XV[35] and the Second Interim Revision Announcement for N F X,[25] is a tracer method and is highly specific for cobalamins which are convertible to cyanocobalamin [36] Many interfering substances, includ ing pseudovitamin B_1, are removed by washing a cresol carbon tetra

chloride solution with dilute sulfuric acid An alumina resin column removes both ionic and nonionic impurities allowing the electrically neutral cyanocobalamin to pass through If treatment with cyanide is omitted the cobalamin assay can be made specific for cyanocobal amin Two hundred to 500 micrograms of total cobalamins are required Although the cobalamin assay requires special equipment and procedures and is rather time-consuming, it is not difficult to apply routinely It is unique in its combination of high accuracy and high precision It can be used to measure cyanocobalamin specifically, even in crude extracts of low potency, as well as cobalamin concen trates liver extracts, and vitamin B_{12} with intrinsic factor concentrates

Colorimetric Assays

Soon after the discovery of crystalline vitamin B_{12} the important role of cyanide in its chemistry was found [37] An assay method based upon the liberation of cyanide from cyanocobalamin by chemical reduction or by photolysis with visible light was developed [38] The cyanide is carefully collected and measured by a highly sensitive colorimetric procedure By measurement of cyanide liberated before and after treatment of the sample with an excess of cyanide ion, this method can be used to determine cyanocobalamin as well as other cobalamins which are readily convertible to cyanocobalamin The colorimetric cyanide method has been used to determine the cyano cobalamin content of many pharmaceutical formulations or the total cobalamin content of liver extracts containing large quantities of substances which interfere with the spectrophotometric measurements of vitamin B_{12} Because it is one of the most sensitive chemical methods for cyanocobalamin it can be used to determine as little as 0 5 microgram with precision Although suitable for assaying formu lations known to contain vitamin B_{12} it is not specific because other non vitamin materials, for example pseudovitamin B_{12}, also liberate cyanide

The dicyanide assay[39] is based upon the difference between the visible spectrum of cyanocobalamin and its purple dicyanide complex formed upon addition of excess cyanide Following preliminary extraction with suitable solvents to eliminate extraneous pigments and to concentrate the vitamin B_{12} this procedure is applicable to the measurements of the total cobalamin content of crude concentrates and culture filtrates

Biological Methods

Biological assay methods for vitamin B_{12} are hampered by the difficulty of satisfactorily depleting the test animals This difficulty is encountered because the young growing animals are endowed by the mother with adequate reserves This has been partially overcome by including stress factors in the diet which intensify the depletion[40, 41] or by the feeding of special diets which increase the requirements for vitamin B_{12} [42, 43] These methods are time-consuming and not highly precise but, with the exception of the anti pernicious anemia test in human subjects, the growth responses in animals are probably the most specific measure of vitamin B_{12} activity and availability

In the chick assay,[44, 45] the chicks used must be deficient in vitamin B_{12} Duplicate groups of chicks are fed vitamin B_{12} free diets supplemented either with known amounts of vitamin B_{12} or with the unknown sample After the assay period, a standard growth response curve is prepared from which the activity of the unknown is estimated

The rat assay[40, 46] uses weanling rats from vitamin B_{12} depleted mothers After seven or more days on a vitamin B_{12}-free diet, the rats receive the test diets for a two week period The assay is based on the average weight gain during this period

Microbiological Methods

The most sensitive methods for detecting and estimating vitamin B_{12} depend on its stimulation of the growth of microorganisms Highly developed methods based on several species of organism have been described [47] The role of vitamin B_{12} in the metabolism of microorganisms has been studied intensively and many vitamin B_{12} like substances have been discovered [48]

Fortunately the problem of specificity is of little concern in the assay of pharmaceutical formulations containing only cyanocobalamin The choice of microbiological assay methods can be made on the basis of precision, experience and convenience, but there is no general agreement on the superiority of any of the many methods

Many of the organisms suitable for assaying cyanocobalamin can be grown either in fluid media in tubes or in agar media on plates, making possible a multitude of permutations In general, plate methods are less sensitive, often by a factor of 1,000 or more, but are easier to use and may be more precise Tube methods using sensitive organisms may measure as little as one micromicrogram of vitamin

B_{12} The preparation of assay samples of most pharmaceutical formulations usually involves simple dilution Extraction of vitamin B_{12} from crude materials and its release from ' bound forms may require special treatment [47]

Lactobacillus Assays

Many methods differing in details of procedure, are based on growth of lactobacilli The vitamin B_{12} activity assay described in U S P XV is a tube assay based on the growth of *Lactobacillus leichmannii* [48] This method has received a great deal of collaborative study and gives results which can be duplicated in different laboratories Although not specific for cobalamins, it is highly sensitive (0 01 millimicrogram per cc) and may be replicated to obtain good precision It is the method of choice for many formulations containing cyanocobalamin The method, as described, measures turbidity due to increased growth, but the Lactobacilli are acid producers and titrimetric procedures for measuring increased acid production can also be used

The earliest microbiological assay method for vitamin B_{12} was based on the growth of *Lactobacillus lactis Dorner* [4] The LLD assays, especially those employing plate methods are still widely used

Escherichia coli Assay

Assays based on the growth of an *Escherichia coli* mutant have been widely used, both by the tube method (sensitivity 0 5 millimicrograms per cc) and the cup plate method (sensitivity 5 0 millimicrograms per cc) [50]

Euglena gracilis Assay

An assay for vitamin B_{12} based on the growth of *Euglena gracilis* has also been developed It can be adapted to tube or plate assay methods with an incubation period of 8 to 10 days [51] It is thought by some to be more sensitive than the *Lactobacillus leichmannii* assay, since as little as one micromicrogram of cyanocobalamin per cc can be determined

Ochromonas malhamensis Assay

An assay based on the growth of the chrysomonad, *Ochromonas malhamensis* which responds almost specifically to cyanocobalamin has been described [52] Because of its slow growth and because few laboratories are familiar with cultures of the organism, this assay method has not come into general use

REFERENCES

1 Minot G R and Murphy W P Treatment of pernicious anemia by a special diet *J A M A* 87 470–476 Aug 14 1926

2 SubbaRow Y, Hastings B A and Elkin M Chemistry of anti pernicious anemia substances in Vitamins and Hormones edited by R S Harris and K V Thimann New York Academic Press 1945 vol 3 p 237

3 Rickes E L Brink N G Koniuszy F R Wood T R and Folkers K Crystalline vitamin B_{12} *Science* 107 396–397 April 16 1948

4 Shorb M S Activity of vitamin B_{12} for the growth of *Lactobacillus lactis Science* 107 397–398 April 16 1948

5 West R Activity of vitamin B_{12} in addisonian pernicious anemia *Science* 107 398 April 16 1948

6 Smith E L Purification of anti pernicious anaemia factors from liver *Nature* 161 638–639, April 24 1948 (in Letters to the Editors)

7 Rickes E L Brink N G Koniuszy F R Wood T R and Folkers K Vitamin B_{12} a cobalt complex *Science* 108 134 Aug 6 1948 (in Comments and Communications)

8 Hodgkin D C Kamper J Mackay M Pickworth J Trueblood K N and White J G Structure of vitamin B_{12} *Nature* 178 64–66 July 14 1956

9 Smith E L Report of Nomenclature Commission (First European Symposium on Vitamin B_{12} and Intrinsic Factor Hamburg 1955) in Vitamin B_{12} and Intrinsic Factor Stuttgart Ferdinand Enke Publisher 1957 pp 554–560

10 Kaczka E A Wolf D E Kuehl F A Jr and Folkers K Vitamin B_{12} XVI Modifications of cyano-cobalamin *J Am Chem Soc* 73 3569–3573 Aug 1951

11 Alicino J F Perchloric acid salt of vitamin B_{12} *J Am Chem Soc* 73 4051 Aug 1951 (in Communications to the Editor)

12 Schmid H Ebnother A and Karrer P Contribution to the knowledge on vitamin B_{12} [in German] *Helvet chim acta* 36 65–75 1953

13 Kuehl F A Jr Shunk C H Moo M and Folkers K Vitamin B_{12} XX 3 3 Dimethyl 2 5-dioxopyrrolidine-4 pr pionamide a new degradation produc *J Am Chem Soc* 77 4418 Aug 20 19 (in Notes)

14 Kaczka E Wolf D E and Folkers K Vitamin B_{12} V Identification of crysta line vitamin B_{12} *J Am Chem Soc* 71 1514–1515 April 1949

15 Beaven G H and Johnson E A The reduction of vitamin B_{12} *Nature* 176 1264–1265 Dec 31 1955 (in Letters to the Editors)

16 Hutchins H H Cravioto P J and Macek T J A comparison of the stability of cyanocobalamin and its analogs in ascorbate solution *J Am Pharm A* (Scient Ed) 45 806–808 Dec 1956

17 Veer W L C Edelhausen J H Wijmenga H G and Lens J Vitamin B_{12} I The relation between vitamins B_{12} and B_{12b} *Biochim et biophys acta* 6 225–228 Sept 1950

18 Armitage J B Cannon J R Johnson A W Parker L F J Smith E L Stafford W H and Todd A R Chemistry of the vitamin B_{12} group Part III The course of hydrolytic degradations *J Chem Soc* 3849–3864 Nov–Dec 1953

19 Brink N G and Folkers K Vitamin B_{12} XVIII The degradation of vitamin B_{12} to 1 α D ribofuranosyl 5 6 dimethylbenzimidazole *J Am Chem Soc* 74.2856–2858 June 5 1952

20 Kaczka E A Heyl D Jones W H and Folkers K Vitamin B_{12} XXI Crystalline α ribazole phosphate and its synthesis *J Am Chem Soc* 74 5549–5550 Nov 5 1952

21 Brink N G and Folkers K Vitamin B_{12} X 5 6-Dimethylbenzimidazole a degradation product of vitamin B_{12} *J Am Chem Soc* 72 4442–4443 Oct 1950

22 Wolf D E Jones W H Valiant J and Folkers K Vitamin B_{12} XI Degradation of vitamin B_{12} to D_g-1 amino-2 propanol *J Am Chem Soc* 72.2820 June 1950 (in Communications to the Editor)

23 Cannon J R Johnson A W and Todd A R Structure of vitamin B_{12} a crystalline nucleotide free degradation product of vitamin B_{12} *Nature* 174 1168 Dec 25 1954

24 Ford J E Holdsworth E S and Kon S K The biosynthesis of vitamin B_{12} like compounds *Biochem J* 59 86–93 Jan 1955

25 Second Interim Revision Announcement National Formulary X *J Am Pharm A* (Pract Ed) 17 725–729 Nov 1956 (Scient Ed) 45 v–ix Oct 1956

26 Hartley F Stross P and Stuckey R E Some pharmaceutical aspects of vitamin B_{12} *J Pharm & Pharmacol* 2 648–659 Oct 1950

27 Macek T J and Feller B A Crystalline vitamin B_{12} in pharmaceutical preparations, *J Am Pharm A* (Scient Ed) 41 285–288 June 1952

28 Gakenheimer W C Pharmaceutical aspects of vitamin B_{12} *Drug & Cosmetic Ind* 72 320–321 and 416 March 1953

29 Bartilucci A and Foss N E Cyanocobalamin (vitamin B_{12}) I A study of the stability of cyanocobalamin and ascorbic acid in liquid formulations *J Am Pharm A* (Scient Ed) 43 159–162 March 1954

30 Blitz, M Eigen E and Gunsberg E Studies relating to the stability of vitamin B_{12} in B-complex injectable solutions *J Am Pharm A* (Scient Ed) 45 803–806 Dec 1956

31 Feller B A and Macek T J Effect of thiamine hydrochloride on the stability of solutions of crystalline vitamin B_{12} *J Am Pharm A* (Scient Ed) 44 662–665 Nov 1955

32 Pharmacopeia of the United States Fifteenth Revision Easton Pa Mack Publishing Company 1955 pp 186–187

33 Mader W J and Johl R G Countercurrent analysis of vitamin B_{12} *J Am Pharm A* (Scient Ed) 44 577–579 Sept 1955

34 Bacher F A Boley A E and Shonk C E Radioactive tracer assay for vitamin B_{12} and other cobalamins in complex mixtures *Analyt Chem* 26 1146–1149 July 1954

35 First Supplement to the Pharmacopeia of the United States Fifteenth Revision Easton Pa Mack Publishing Company 1956 pp 21–23

36 Bruening C F Neuss J D Numerof P and Kline O L Cobalamin assay by the radioisotope tracer method a collaborative study report of the U S P N F joint panel *J Am Pharm A* (Scient Ed) 46 66–72 Jan 1957

37 Wolf F J Process for recovering vitamin B_{12} U S Patent No 2 530 416 Nov 21 1950

38 Boxer G E and Rickards J C Chemical determination of vitamin B_{12} II The quantitative isolation and colorimetric determination of millimicrogram quantities of cyanide *Arch Biochem* 30 372–381 Feb 1951

39 Rudkin G O Jr and Taylor R J Chemical method for determining vitamin B_{12} *Analyt Chem* 24 1155–1156 July 1952

40 Bosshardt D K Paul W J O Doherty K Huff J W and Barnes R H Mouse growth assay procedures for the animal protein factor *J Nutrition* 37 21–35 Jan 1949

41 Nichol C A Dietrich L S Cravens W W and Elvehjem C A Activity of vitamin B_{12} in the growth of chicks *Proc Soc Exper Biol & Med* 70 40–42 Jan 1949

42 Cuthbertson W F J and Thornton D M The assay of vitamin B_{12} 7 Effect of dietary lactose and of the state of maternal nutrition on the growth response of the rat to vitamin B_{12} *Brit J Nutrition* 6 170–175 1952

43 Hill D C and Branion H D Influence of diet composition on the response of chicks to vitamin B_{12} *Poultry Sc* 31 892–897 Sept 1952

44 Ott W H Further studies of the activity of crystalline vitamin B_{12} for chick growth *Poultry Sc* 30 86–91 Jan 1951

45 Coates M E Harrison G F and Kon S K The measurement of vitamin B_{12} by biological assay with chicks *Biochem J* 46 vii–viii 1950 (in Soc Proc)

46 Register U D Ruegamer W R and Elvehjem C A An improved assay for a growth factor in liver extracts *J Biol Chem* 177 129–134 Jan 1949

47 Hoff Jorgensen E Microbiological Assay of Vitamin B_{12} in Methods of Biochemical Analysis New York Interscience Publishers Inc 1954 vol 1 pp 81–113

48 Ford J E and Hutner S H Role of Vitamin B_{12} in the Metabolism of Micro organisms in Vitamins and Hormones New York Academic Press Inc 1955 vol 13, pp 101–136

49 *Pharmacopeia of the United States Fifteenth Revision* Easton Pa Mack Printing Company 1955 pp 885–889

50 Davis B D and Mingioli E S Mutants of *Escherichia coli* requiring methionine or vitamin B_{12} *J Bact* 60 17–28 July 1950

51 Hutner S H Provasoli L Stokstad E L R Hoffman C E Belt M Franklin A L and Jukes T H Assay of anti pernicious anemia factor with *Euglena* *Proc Soc Exper Biol & Med* 70 118–120 Jan 1949

52 Ford J E The microbiological assay of vitamin B_{12} The specificity of the requirement of *Ochromonas malhamensis* for cyanocobalamin *Brit J Nutrition* 7 299–306 1953

Part 2

Nutritional, Metabolic, Physiologic, Pharmacologic and Clinical Information

THE NUTRITIONAL ROLE OF VITAMIN B_{12}

Vitamin B_{12} has been found to be essential for the growth of many microorganisms Among these microorganisms are *Lactobacillus lactis Dorner*[1] *Lactobacillus leichmannii*,[2] a mutant strain of *Escherichia coli*,[3] the chlorophyll-containing protozoan, *Euglena gracilis*[4] and the chrysomonad, *Ochromonas malhamensis*[5]

Of the vitamin B_{12} dependent microorganisms known, the lactic acid bacteria are the most sensitive and *Ochromonas malhamensis* the most specific in their requirement for vitamin B_{12} Methionine, desoxyribosides, or pseudovitamin B_{12} are capable of supplying the vitamin B_{12} requirements for most of these organisms None of these compounds are active, however, for *Ochromonas malhamensis*

Table 1 The Vitamin B_{12} Activity Content of Some Common Foods

Food	Vitamin B_{12} Activity (mcg /Kg)
Milk products	
Whole milk	3 5 (per liter)
Evaporated milk	1 3 (per liter)
Condensed milk	3 5 (per liter)
Whole milk powder	10 26
Skim milk powder	25 40
Cheese	
American	6
Swiss	9
Cream	2
Canned meats	
Beef with juice	10 25
Pork with juice	10 20
Veal with juice	10 20
Lamb with juice	20 50
Beef liver	200 600
Cured meats	
Bologna sausage	17
Pressed ham	11
Frankfurter	18
Pork sausage	15
Sea foods	
Fillet of ole	13
Haddock	6
Scallops	7
Eggs (whole)	1 5 per egg

In the following foods microbial growth response was equivalent to less than 1 mcg per Kg of Vitamin B_{12} activity Canned carrots beets peas green beans canned soups—tomato vegetable chicken lamb vegetable cereal products—white flour whole wheat flour white bread

Not only do some microorganisms require vitamin B_{12}, but many are capable of synthesizing relatively large quantities of the vitamin[6] Cobalt markedly stimulates the biosynthesis of the vitamin by micro organisms grown in common bacteriologic media[7] Detectable quantities of vitamin B_{12} activity are found in animal proteins and fermentation products Plant proteins appear to be devoid of the vitamin Meats, especially liver, and eggs, milk, cheese and fish are among the richer sources of the vitamin Table 1 indicates the content of the vitamin B_{12} activity in several groups of common foodstuffs[8] The vitamin B_{12} activity content of these foods was determined by microbial assays employing the lactic acid bacteria Although these micro organisms respond to materials other than vitamin B_{12}, the values obtained agree fairly well with those obtained by other methods

During the early investigations on liver and its relation to pernicious anemia, nutritionists also were engaged in determining the nutritional requirements of domestic animals Studies on chicks and rats firmly established that optimal growth in these species was a function of animal protein intake The essential nutrient present in animal protein was designated animal protein factor (APF)[9] Vitamin B_{12} was shown in chick experiments to possess a high degree of APF activity[10] Vitamin B_{12} was also found essential for normal growth of the rat,[11] the mouse[12] and the pig[13]

It is now firmly established that vitamin B_{12} is an essential growth factor for certain microorganisms, for laboratory animals, and for poultry and nonruminant farm animals Although it is also essential to human nutrition, the minimum daily requirements of vitamin B_{12} have not been established

THE METABOLIC ROLE OF VITAMIN B_{12}

Although the physiologic effects of vitamin B_{12} on blood formation, neural function and the maintenance of growth in young animals are now well established, these effects cannot as yet be fully explained in terms of specific metabolic functions of the vitamin Extensive data have been collected, however, on the influence of vitamin B_{12} on a number of biochemical reactions which are concerned with intermediary metabolism

Indirect evidence indicates that vitamin B_{12} plays a role in the synthesis of nucleic acids Vitamin B_{12} deficiency in rats decreases the

ribonucleic acid content of the liver, spinal cord and cervical ganglia [14] Although both the ribonucleic and desoxyribonucleic acids per gram of liver are significantly reduced in the deficient animals,[14, 15] there is no change in the amount of nucleic acid per cell [15] It appears, therefore, that the synthesis of nucleic acid is markedly reduced in vitamin B_{12} deficiency and this causes a decrease in cell division The effect of vitamin B_{12} deficiency on nucleic acid synthesis and cell division has also been demonstrated in human bone marrow cells from pernicious anemia patients [16] Considerable decrease in the synthesis of thymine was observed in marrow cells, which is consistent with other data on the specific function of vitamin B_{12} in the synthesis of this pyrimidine base [17]

Rats receiving vitamin B_{12} will grow on diets deficient in choline and methionine, but containing homocystine This suggests that the vitamin has a function in the metabolism of labile methyl groups [18] Further evidence of this function is provided by the observation that vitamin B_{12} will prevent the characteristic hemorrhagic kidney syndrome which develops in weanling rats fed a choline free diet [19] Studies with vitamin B_{12}-deficient rats and baby pigs fed precursors con taining C^{14} labeled methyl groups have shown that vitamin B_{12} enhances the synthesis of choline and methionine methyl groups from formate, from the β-carbon of serine and the α-carbon of glycine [20-22] The available evidence also shows, however that vitamin B_{12} is not involved in the actual mechanism of transmethylation from betaine to methionine and from methionine to choline [23-25]

Vitamin B_{12} deficiency in rats, as well as in patients with pernicious anemia, causes a marked diminution in the blood levels of soluble sulfhydryl compounds [26] The glutathione content of liver and blood of vitamin B_{12}-deficient rats is significantly diminished [27] Some of the effects of vitamin B_{12} on carbohydrate metabolism may be mediated through glutathione, which as the prosthetic group of glyceraldehyde-3 phosphate dehydrogenase, is presumably essential in the glycolytic pathway Homocysteine, on the other hand, is essential as an acceptor of methyl groups in the process of labile methyl group synthesis and transfer [18]

Vitamin B_{12} deficiency in rats and chicks leads to a marked increase in the coenzyme A content of the liver and kidneys [28, 29] The increase occurs chiefly in the sulfhydryl (reduced) form of the coenzyme The increase is probably a physiologic adaptive mechanism and may be

involved in the effects of vitamin B_{12} deficiency on carbohydrate and fat metabolism [30]

It has been postulated that vitamin B_{12} may be involved in the conversion of folic acid to folinic acid [31] It has been shown that this conversion *is decreased in liver homogenates from vitamin B_{12} deficient* hens [32] There is also some indication that the conversion of folic acid to folinic acid is decreased in pernicious anemia patients in relapse [33] Since the conversion of folic acid to folinic acid by chick liver homogenates is enhanced by homocysteine,[34] it is possible that the action of vitamin B_{12} is an indirect one which concerns the increased reduction of homocystine to homocysteine

On the basis of growth studies in animals it has been shown that vitamin B_1 has some general effects on animal metabolism In hyperthyroid rats, it has a protein sparing action This does not prevent *weight loss, however, for protein is spared at the expense of other* body constituents [35] Vitamin B_{12} also permits better utilization of nitrogen in animals fed a diet high in soybean protein [36 37] Since the methionine content of soybean protein is low, this effect is probably related to the methionine sparing activity of the vitamin rather than to a direct effect of vitamin B_{12} on protein synthesis

Some of the increased growth of animals receiving vitamin B_{12} has been ascribed to an increase in appetite [38 39] This view is supported by the finding that the protein catabolic action of cortisone in rats is counteracted by vitamin B_{12} under ad libitum feeding conditions,[39] *but is not affected when food intake is restricted* [40] *Most of* the increase in weight gain in young rats fed a plant protein diet supplemented with vitamin B_{12} can be accounted for by gain of fat rather than protein [41]

Some recent investigations in animals indicate that vitamin B_{12} is concerned with the metabolism of carbohydrates It has been shown that the catabolism of fructose by rat liver homogenates is enhanced in animals receiving vitamin B_{12} [42] It has also been shown in rats that the feeding of lactose[43] or the injection of glucose[44] leads to an increased requirement for vitamin B_1 Vitamin B_{1} deficiency in chicks leads to an increase in blood glucose, while the increase in blood glucose obtained by feeding high levels of glycine, glutamic acid or methionine is enhanced by the administration of vitamin B_{12} This suggests that the vitamin may function in the conversion of certain glucogenic amino acids into glucose [45]

Vitamin B_{12}-deficient rats on a high-carbohydrate, low fat diet rapidly lose weight, develop hyperglycemia and show a decrease in carcass phospholipids, which suggests that these animals have partially lost their ability to transform carbohydrates into lipids In accord with this suggestion is the observation that abnormally small amounts of phospholipids are found in the tissues of vitamin B_{12}-deficient rats and in the blood of patients with pernicious anemia in relapse [41] The utilization of high dietary fat intake in chicks is improved following the administration of vitamin B_{12},[46] indicating that vitamin B_{12} may be required for the metabolism of fat

Although many investigations have shown that vitamin B_{12} has a marked influence on animal growth, and certain evidence indicates that the vitamin aids in the utilization of proteins, carbohydrates and fats, the exact mechanism by which this is accomplished is still unknown Experiments in rats have shown that vitamin B_{12} will counteract the effects of excess production of or the administration of thyroid hormone[17] and adrenocortical hormones [48] It has not been determined, however, whether there is a direct physiologic relation between vitamin B_{12} and these hormones or whether these observed effects are due to the indirect influence of the vitamin on some phases of intermediary metabolism

PHYSIOLOGY AND PHARMACOLOGY OF VITAMIN B_{12}

Vitamin B_{12} is now known to be identical with the extrinsic or erythrocyte maturing factor of Castle,[49] which is essential for normal bone marrow erythropoietic activity in man In the absence of the erythrocyte maturation factor, as in pernicious anemia and other macrocytic anemias red cell maturation progresses abnormally and is arrested at a megaloblastic stage of development Vitamin B_{12} is also concerned with neural function and the maintenance of nerve cells and is required to sustain normal growth in young animals The sources of vitamin B_{12} for human nutrition are foods of animal origin such as meats, milk and eggs

Vitamin B_{12} and Intrinsic Factor

Vitamin B_{12} in foods is very poorly absorbed by human beings unless intrinsic factor is present at the same time Extensive studies[49-51] have shown that an extrinsic factor present in the diet, which is now

known to be a vitamin B_{12} active substance, is required for normal hemopoiesis in man Intrinsic factor, produced in the stomach, is found in abundance in normal gastric juice Its function is to facilitate the passage of vitamin B_{12} across the intestinal mucous membrane Pernicious anemia occurs in human beings who lack intrinsic factor and, therefore, are unable to absorb vitamin B_{12} from dietary sources in amounts adequate for normal hemopoietic function Although considerable progress has been made in attempts to isolate and purify the intrinsic factor,[52–55] its chemical structure and the exact nature of its action in facilitating the absorption of vitamin B_{12} are still unknown [56]

It was originally postulated that the intrinsic factor interacted in some manner with vitamin B_{12}, probably in the small intestine, and that the product of this interaction was present in blood serum as hemopoietic factor [57] Despite extensive studies to determine the nature of the interaction between vitamin B_{12} and intrinsic factor, information is still incomplete concerning the exact site of the interaction, if any, and the conditions controlling it

Some species specificity of intrinsic factor is indicated by studies which showed that intrinsic factor of human or porcine origin, which is active in pernicious anemia patients, did not facilitate the intestinal absorption of vitamin B_{12} in gastrectomized rats, but rat intrinsic factor was fully active [58, 59]

Absorption and Excretion of Vitamin B_{12}

If the amounts of vitamin B_{12} ingested are small and intrinsic factor is present, most of the absorption takes place with the aid of this factor [60] Over a certain range, there is a stoichiometric relationship between the amount of vitamin B_{12} absorbed by pernicious anemia patients and the amount of intrinsic factor given [61] When large amounts of vitamin B_{12} are given, however, some direct intestinal absorption of the vitamin occurs without mediation of intrinsic factor The direct absorption assumes greater importance as the level of intake of vitamin B_{12} is increased [62] The amount absorbed by direct diffusion across the intestinal barrier, however, is very small when relatively large amounts of the vitamin are administered orally to normal individuals or to pernicious anemia patients receiving adequate intrinsic factor [62, 63] The intestine has a limited capacity to absorb vitamin B_{12} given in single oral doses even in the presence of an excess of intrinsic factor [64] The amount of vitamin B_{12} absorbed and

the level at which intestinal absorption is restricted varies greatly in normal subjects and in patients with pernicious anemia [61 62 63]

After rectal administration of very large doses, the increase in serum concentrations and urinary excretion of vitamin B_{12} and the hematologic responses in pernicious anemia patients indicate significant but variable absorption [62] Direct absorption of vitamin B_{12} by the nasal mucosa following the instillation or insufflation of large therapeutic doses in pernicious anemia patients has been observed,[64 67] and the urinary excretion patterns are similar to those observed after parenteral injections [65]

Vitamin B_{12} containing radioactive cobalt has been used quite successfully, in tracer studies to estimate the intestinal absorption of the vitamin [65] When the oral dose of labeled vitamin B_{12} is very small (0 5 microgram) more than 50 per cent of the administered dose is absorbed in normal persons and less than 15 per cent in pernicious anemia patients, as determined by fecal elimination and hepatic deposition of radioactive cobalt [69] When the oral dose of labeled vitamin B_{12} is increased to about 5 0 micrograms, as much as 70 to 80 per cent of the radioactivity may be eliminated in the feces, indicating very little absorption even in normal persons [70 71]

The absorption of physiologic amounts of the vitamin has been similarly estimated by measuring over a fixed period the radioactivity of the urine following oral administration of small doses of radioactive vitamin B_{12} The radioactive vitamin B_{12} is flushed from the body by a subsequent large intramuscular dose of nonradioactive vitamin B_{12} Under these conditions all of the radioactivity found in the urine must be derived from the vitamin B_{12} absorbed from the gastrointestinal tract [7] Renal disease must be considered in interpretation of the results of this test since individuals with kidney involvement may show a low rate of vitamin B_{12} excretion, sometimes in the range shown by pernicious anemia patients Such individuals, however, unlike pernicious anemia patients, do not show increased excretion with administration of intrinsic factor, and they continue to excrete the radioactive vitamin beyond the first 24 hours after the flushing dose [73]

Labeled vitamin B_{12} has also been used to study the distribution and excretion of vitamin B_{12} after parenteral and oral administration to rats After subcutaneous injection, approximately half of the radioactivity was rapidly excreted in the urine, and about 6 per cent in the

feces The remainder was distributed throughout the body in various organs After oral administration, 81 per cent was eliminated in the feces, and about one per cent in the urine [74]

After absorption, vitamin B_{12} appears in the blood stream and circulates to the various sites of its metabolic activity Normal human serum levels of vitamin B_{12} vary greatly and may range from 100 to 900 micromicrograms per cc [63] *with the major portion apparently bound* to the alpha globulin fractions of the serum proteins [75] The serum concentrations in patients with pernicious anemia are definitely below the normal range After treatment with vitamin B_{12}, the serum concentrations return to normal or above and such levels can be maintained by adequate therapy When therapy is discontinued, the serum concentration of vitamin B_{12} gradually declines as relapse occurs The bone marrow, however, usually remains normoblastic as long as the serum levels are above 100 micromicrograms per cc [69]

Parenterally administered vitamin B_{12} is rapidly absorbed and appears in the blood serum in both free and bound form Following single injections in normal persons or in patients with pernicious anemia in relapse, serum levels of vitamin B_{12} rise rapidly, reach a maximum within one hour and decline gradually over a period of 24 hours or more Most of the injected vitamin is rapidly excreted in the urine The proportion of the dose excreted increases with the amount injected [76] The free form of the vitamin is readily excreted by the kidney

Following single oral doses of vitamin B_{12}, little or no change in serum levels has been observed unless the amount given is 500 micrograms or more [63] With single oral doses of 1,000 to 3,000 micrograms or more, definite but variable increases in the serum concentrations have been demonstrated [62, 76] After these large oral doses there is an increase in urinary excretion of the vitamin which is considerably less than that observed after intramuscular injection of amounts that produce equivalent serum concentrations [76] This lower urinary excretion is associated with greater binding of the vitamin by serum protein after oral than after parenteral administration [62]

Toxicity

Although vitamin B_{12} is probably the most active biological principle known, it is not toxic when administered orally or parenterally No manifestations of toxicity were observed in studies with mice, rats and guinea pigs Intraperitoneal or intravenous injections of crystal

line vitamin B_{12} in quantities as high as 1.6 Gm. per kilogram in mice produced no toxic effects. No toxic effects were produced in rats and guinea pigs with intraperitoneal injections of 100 mg. per kilogram.[77]

No acute or chronic toxic effects have been observed in man even after the parenteral administration of very large doses of pure crystalline vitamin B_{12}. Doses as large as 1,000 micrograms or more have been administered by subcutaneous or intramuscular injection without local irritation.[60] Daily intravenous injection of doses as large as 3,000 micrograms were painless and nontoxic and no allergic side reactions were observed.[78] Single oral doses as large as 10,000 micrograms administered to pernicious anemia patients have produced no apparent toxic effects.[9] During continued oral therapy with doses of 1,000 micrograms of crystalline vitamin B_{12} per week for periods of three to five years, no adverse effects were observed.[80]

Although a few allergic reactions were observed following the therapeutic use of some of the early concentrates[81] and crystalline preparations of vitamin B_{12},[60] hypersensitivity reactions have not occurred following administration of the pure crystalline vitamin.[80] In a group of patients who showed allergic reactions after therapeutic liver injections, with strong positive reactions to liver extracts, skin tests with pure vitamin B_{12} were negative.[82]

CLINICAL USES

Since its discovery, vitamin B_{12} has been found useful in an increasing number of clinical conditions. Its efficacy in pernicious anemia is established beyond question and it has often been effective in other macrocytic anemias. It has also found a place in the treatment of various neurologic disorders. These include not only the neurologic disturbances accompanying pernicious anemia and diabetes mellitus but also trigeminal neuralgia, herpetic neuralgia and possibly other neuropathies.

Other clinical studies indicate the potential usefulness of the vitamin in a variety of physiologic states or clinical conditions. Certain skin disorders and liver disorders have responded in some cases. The increased need for vitamin B_{12} in many nutritional states is becoming increasingly apparent. It is being used as a dietary supplement in debilitated or elderly patients, and it has been useful in promoting growth in ill or undernourished children. In such conditions the beneficial effects of the vitamin on appetite have been extremely important.

Extensive investigation has indicated that vitamin B_{12} is effective not only by the parenteral route but also in large oral doses and, in some cases, by nasal insufflation. Pure crystalline vitamin B_{12} does not produce local irritation or sensitivity reactions when administered parenterally, nor does it cause gastric irritation when given orally.

Macrocytic Anemias

Vitamin B_{1}. deficiency is involved in certain anemias resulting from disturbance of the hemopoietic function of the bone marrow. If the vitamin is lacking in the diet for a prolonged period, or if it is inadequately absorbed or utilized, erythrocyte maturation is arrested, resulting in macrocytosis and a decline in red cell count. In addition, megaloblasts may appear in the bone marrow and peripheral blood. Megaloblastosis is characteristic of pernicious anemia and occurs frequently in the other macrocytic anemias in which vitamin B_1 deficiency is involved.

Following vitamin B_{12} therapy in such anemias, the hemopoietic functions of the bone marrow are promptly restored to normal. Erythrocyte maturation is hastened, the per cent of reticulocytes in the peripheral blood is increased temporarily, megaloblasts are greatly reduced in number, and are replaced by more mature cells. The red cell count gradually returns to normal.

Addisonian Pernicious Anemia

Pernicious anemia is a chronic, relapsing disease, caused by a conditioned deficiency of vitamin B_1.[83] It is a fatal disease unless the deficiency is corrected. The deficiency results from failure of the gastric mucosa to produce sufficient intrinsic factor,[51] the substance essential for absorption of extrinsic factor, which is now known to be vitamin B_{12} or a B_{12} active substance. Patients lacking intrinsic factor are unable to assimilate the vitamin even when it is present in normal amounts in the diet. The amount of intrinsic factor present in pernicious anemia patients probably varies,[84] but it is inadequate for absorption of the required amounts of vitamin B_{12} in all these patients.[83] The resulting disease involves primarily the hemopoietic system, the nervous system, and the alimentary tract. Characteristic pathologic changes involve the tongue, gastric mucosa, bone marrow, peripheral blood, and nervous system. Achlorhydria, a characteristic finding, is evidence of the defect in the gastric mucosa which causes the disorder.

The red cell count is reduced, in some cases to less than 10 per cent of normal Although hemoglobin synthesis is not affected the great reduction in red cells means that the percentage of hemoglobin present in the blood is also greatly reduced The average diameter of the erythrocytes is increased and many very large cells called macrocytes are found Reticulocytes are increased to about 2 or 3 per cent of the red cell population Megaloblasts are characteristically present in the blood The bone marrow is megaloblastic and hyperplastic

Neurologic changes range from peripheral nerve degeneration to degeneration in the posterior and pyramidal tracts of the spinal cord and in the subcortical areas in the motor region of the brain Such changes lead to a variety of symptoms, including paresthesias difficulties in walking incoordination, loss of position sense, and mental disturbances

Other common symptoms of the disease include weakness, anorexia, pallor, and gastrointestinal disturbances Soreness of the tongue which may become smooth because of papillary atrophy, is common Liver and spleen enlargement are sometimes present The skin may take on a lemon yellow tint

The treatment of choice is vitamin B_{12}, which promptly produces complete hematologic remission and neurologic improvement [85] The first detectable response is a dramatic increase in the number of reticulocytes which rise to a maximum within a week after the start of therapy Thereafter the reticulocytes rapidly mature and the erythrocyte count gradually rises Erythrocytes and hemoglobin usually are normal within four to six weeks Bone marrow is converted from a megaloblastic to a normoblastic state within 48 to 72 hours Within a few days after start of treatment appetite and sense of well being improve Glossitis subsides and after several weeks lingual papilla regeneration takes place Neurologic complications respond more *slowly to vitamin B_{12} therapy but will subside* under continued treatment unless irreversible changes in the brain or spinal cord have occurred [83 86 87] (The neurologic complications of pernicious anemia and their response to vitamin B_{12} therapy are further discussed under NEUROLOGIC DISORDERS) Patients with pernicious anemia can be maintained in good health as long as adequate vitamin B_1 therapy is continued [87 88]

The efficacy of vitamin B_1 administered by the parenteral route for pernicious anemia has long been established It is also effective when

given orally with intrinsic factor [63] One study in pernicious anemia patients, however, has indicated that this method may lose its efficacy after prolonged use of heterologous intrinsic factor, though patients will still respond satisfactorily if human intrinsic factor is used The authors postulate that some failures with oral treatment may be due to this phenomenon [89]

Formerly it was thought that intrinsic factor was essential to effect absorption of orally administered vitamin B_1 [84 90 91] Investigation has shown, however, that even without intrinsic factor, very large oral doses may produce a satisfactory hematologic and neurologic response which can be sustained by continued therapy [87 9 93]

Anemia Following Gastrectomy

Failure to secrete intrinsic factor with resultant lack of vitamin B_{12} absorption not only occurs in pernicious anemia but may also follow gastrectomy or accompany malignant disease of the stomach Consequently a macrocytic anemia closely resembling pernicious anemia[94-96] frequently follows gastrectomy and is sometimes associated with gastric carcinoma In the latter case this may be the result of interference with intrinsic factor secretion by the carcinomatous growth Some investigators, however, suggest that lack of intrinsic factor may precede rather than follow development of gastric cancer [97] In this connection the increased incidence of the disease in pernicious anemia patients and in nonanemic but achlorhydric patients has been reported and the authors believe that patients with achylia gastrica, as demonstrated by the Schilling urinary excretion test, probably have the same predilection for gastric cancer as those with pernicious anemia [97]

In totally gastrectomized patients, the reason for failure to secrete intrinsic factor is loss of the fundal portion of the stomach [85] Tests with small doses of radioactive vitamin B_1 show that this amount of the vitamin is not absorbed when administered to patients with total gastrectomy unless intrinsic factor is also administered [98 89] Consequently in untreated patients the concentration of vitamin B_{12} in the serum decreases progressively after operation [98] In spite of this, the anemia usually does not appear for several years following the operation and neurologic symptoms may be even longer delayed [100] Occasionally, spinal cord degeneration develops,[101] even when anemia is not present [10] Both anemia and neurologic symptoms in these patients respond well to treatment with vitamin B_1 [100 103,104] Whether total

gastrectomy is done for the treatment of carcinoma or peptic ulcer, vitamin B_{12} should thereafter be administered regularly to prevent the development of macrocytic anemia, which otherwise frequently follows [96, 98, 100, 103]

Macrocytic anemia has also been reported after partial gastrectomy and after gastroenterostomy,[104-107] though its incidence is not as high as it is following complete gastrectomy [107] Regular administration of vitamin B_{12} is therefore advisable following these operations

Anemia Accompanying Intestinal Strictures and Anastomoses

A macrocytic anemia which responds to vitamin B_{12} is occasionally associated with intestinal strictures or anastomoses [108, 109] Symptoms are like those in pernicious anemia and the blood findings are identical with those in pernicious anemia both in morphology and degree of reduction of the red cell count The bone marrow is hyperplastic and megaloblastic Achlorhydria however, is not characteristic and intrinsic factor is usually present Although the exact mechanism of the production of this anemia is not known, it has been postulated[110] that intestinal stagnation due to stricture permits abnormal bacterial growth in the small intestine, or short circuit of the intestine occurs, either of which may result in impaired utilization of vitamin B_{12} Relief of symptoms and satisfactory hematologic response have been observed in this type of macrocytic anemia following the administration of vitamin B_{12}, whether or not the intestinal anomaly has been corrected by surgical measures

Fish Tapeworm Anemia

The symptoms and hematologic findings in some patients infested with fish tapeworm *(Diphyllobothrium latum)* are almost indistinguishable from those in pernicious anemia [111] The macrocytic anemia is caused by subnormal absorption of vitamin B_{12}, or by competitive use of the vitamin by the parasite [111] Although removal of the worm is the primary therapy, vitamin B_{12} produces satisfactory hematologic and neurologic responses whether or not the worm has been passed [84, 111, 113]

Nutritional Macrocytic Anemias

The symptoms of nutritional macrocytic anemia, including glossitis and associated mucous membrane lesions, are similar to those in

pernicious anemia, and the hematologic findings are identical Severe neurologic disturbances, however, are rare, also deficient or otherwise abnormal gastric secretions are not characteristic of the condition [85] The syndrome is encountered in malnourished persons

A deficiency of either vitamin B_{12}, or more frequently folic acid, or both, is the cause of this anemia [85] Individuals who eat much carbo hydrate but little protein or fat, either because of poverty, social habits, or religious beliefs, are subject to such deficiencies The anemia is frequently found in association with other severe deficiency states such as beriberi and pellagra Macrocytic anemias resulting from deliberate deprivation of dietary sources of vitamin B_{12} have also been reported Among the best examples of this are the Vegans, who eat no animal products During the period of deprivation of vitamin B_1, their serum vitamin B_1 levels fall and they eventually develop pares thesias, menstrual disorders, pains of the spine and back, and finally anemia The addition of vitamin B_1 to the diet alleviates these manifestations [114-117]

A study of the serum vitamin B_1 levels in 6 patients with nutri tional macrocytic anemia in India revealed that the values were signifi cantly lower than in normal Indians [118] Some African patients, on the other hand, showed high serum vitamin B_1 levels and subsequently responded only to folic acid therapy [119]

Vitamin B_1 given parenterally may produce a hematologic re sponse in this disease and is advisable as the initial treatment if the diagnosis is doubtful [120] The oral route may be satisfactory but paren teral administration is essential when gastrointestinal absorption has been impaired [85]

In many patients deficiency of vitamin B_1 and folic acid may be present simultaneously In such patients administration of both vita- mins may lead to more favorable results than would the administra- tion of either vitamin alone It is advisable to select vitamin B_{12} as the initial therapeutic agent in cases in which the diagnosis is uncertain [83]

A megaloblastic anemia of nutritional origin is occasionally en countered in infants 3 to 18 months old This disease has almost dis appeared in the United States as a result of vitamin supplementation and better dietary habits It occurs in children maintained entirely on a diet of goat s milk or of powdered cow's milk[121] and in those whose diets were not supplemented by required foods at the proper time [12] Loss of appetite, intermittent fever, and pallor characterize the disease

Hemic murmurs hepatic enlargement and, less consistently, splenomegaly may be found The blood findings are identical with those in pernicious anemia except that leukocytosis may be present

The etiology of this anemia is not certain but it is probably due to a multiple vitamin deficiency[123] In some studies vitamin B_{12} alone has produced satisfactory response,[122,124] but frequently it has been ineffectual and therefore should not be used as the sole therapeutic agent[85,125]

Dimorphic anemias of nutritional origin in which a deficiency of vitamin B_{12} or folic acid and a deficiency of iron coexist, may be macrocytic and hypochromic An initial response may be obtained following administration of vitamin B_{12} or folic acid but the peripheral blood findings may then change to those of a microcytic, hypochromic anemia Although separate deficiencies of both vitamin B_{12} and iron may thus be demonstrated, an erythropoietic response to iron therapy may not occur unless vitamin B_{12} is also administered

Vitamin B_{12} is not indicated in the specific therapy of hemolytic anemia or the anemia of chronic blood loss, but it may be administered if the anemia is so prolonged that severe secondary symptoms such as anorexia, nausea, and impaired metabolic function are produced, thus creating a conditioned nutritional deficiency of vitamin B_{12}

Anemias Associated with Gastrointestinal Disorders

Tropical and nontropical sprue, idiopathic steatorrhea and celiac disease are chronic relapsing diseases characterized by voluminous frothy stools and frequently by macrocytic anemia and a megaloblastic bone marrow Neurologic changes may occur which are similar to, but less severe than those found in pernicious anemia The etiology of these conditions is not fully understood, but in all of them gastrointestinal disturbances and voluminous frothy stools occur, resulting in impairment of vitamin B_{12} absorption[85,126]

Vitamin B_{12}, administered parenterally in doses larger than those given in pernicious anemia, has been successfully used in the treatment of these disorders[85,86,127,128] These disorders are characterized by deficiencies of both folic acid and vitamin B_{12} and both hemopoietic agents have been used in their treatment[129] The parenteral route has been recommended as the most effective and practical for the administration of vitamin B_{12}[130] since gastrointestinal function is impaired Oral doses of vitamin B_{12} however have been reported effective in some cases[131,132]

Anemia of Pregnancy

In megaloblastic anemia of pregnancy, the blood and marrow changes are usually very similar to those in pernicious anemia Lesions of the spinal cord are absent, however, and achlorhydria is not a consistent finding The onset of the anemia is usually in the third trimester It has been observed most frequently in women whose diets have been deficient, but does occur in women on apparently adequate diets often enough to raise some doubts as to its dietary origin [8]

This anemia is far more serious than the iron deficiency anemia of pregnancy or the physiologic anemia due to increased plasma volume The megaloblastic anemia occurs far less frequently than the other two types There is, however, evidence that cases of hypochromic anemia in malnourished women may be accompanied by a macrocytosis due to vitamin B_{12} deficiency In one study of 100 anemic pregnant patients, living under depressed economic conditions with poor nutrition, 11 had hyperchromic macrocytic anemia, 30 had hypochromic microcytic anemia, and 59 had hypochromic anemia accompanied by macrocytosis Though typical megaloblasts were found in only a few of these cases, the bone marrow was hyperplastic and contained giant stab cells and giant myelocytes In most of the patients who developed this dimorphic anemia, the diet was protein deficient, and both the serum iron and vitamin B_{12} concentrations were low The vitamin B_{12} levels, in fact, approached those in patients with megaloblastic anemia The authors suggest that the typical morphologic picture of megaloblastic anemia failed to develop in these patients only because of the concomitant iron deficiency [133]

Vitamin B_{12} administered alone has produced striking hemopoietic responses in cases of megaloblastic anemia of pregnancy associated with nutritional deficiency [134 135] It has also been effective in combination with ascorbic acid, which has proved ineffective alone [136] Folic acid has been more consistently effective than vitamin B_{12} [85,137] However, even when folic acid deficiency is the chief causative factor, vitamin B_1 may enhance response to folic acid therapy and hasten recovery

NEUROLOGIC DISORDERS

Because the neurologic manifestations of pernicious anemia frequently respond dramatically to vitamin B_1 many investigators have treated other neurologic disorders with this vitamin Thus far, vitamin B_{12} has

proved useful in the treatment of several neuropathies, including trigeminal neuralgia postherpetic neuralgia, diabetic neuritis, and other painful syndromes of obscure etiology [138] The results, though difficult to evaluate because of the subjective nature of the symptoms, were prompt and definite enough in some instances to indicate that the vitamin has a direct action on nerve tissue

Neurologic Complications of Pernicious Anemia

From 70 to 95 per cent of patients with pernicious anemia manifest varying degrees of neurologic involvement [139] The symptoms are primarily those of sensory tracts and nerves Peripheral nerve degeneration causes numbness and tingling, and sometimes hyperesthesia of the soles of the feet Damage to the posterior and lateral columns of the spinal cord can cause ataxia, absence of reflexes, and incoordination Changes in motor nerves, which are now rare, can cause paralysis and muscular atrophy

Mental changes also occur in many patients These include irritability, impaired concentration, dullness, drowsiness, and depression It has been pointed out in this connection that cerebral as well as spinal lesions accompany pernicious anemia, but are often unrecognized The end result, if treatment is inadequate, may be a severe dementia [140] Cerebral symptoms may precede the appearance of anemia or of spinal or peripheral nerve involvement

Vitamin B_{12} is essential for the prevention and treatment of the neurologic and mental complications of pernicious anemia Prompt improvement occurs and complete relief can eventually be obtained with intensive vitamin B_{12} therapy, except when treatment has been delayed until irreversible changes have taken place Adequate vitamin B_{12} maintenance therapy also will prevent neurologic relapse [63 86 87]

In connection with the neurologic manifestations of pernicious anemia, it should be emphasized that folic acid not only is less effective hemopoietically in pernicious anemia than liver extract or vitamin B_{12}, but it also fails completely to alleviate or prevent the neurologic complications An even more important consideration is that the transitory hemopoietic effect of folic acid therapy may dangerously mask progression of the disease It has become increasingly common to find pernicious anemia patients who, having been treated with folic acid alone or in conjunction with insufficient vitamin B_{12} show a temporarily satisfactory hematologic response but then suddenly develop

severe neurologic symptoms [63] It has been suggested that by stimulating hemopoiesis when an acute vitamin B_{12} deficiency exists, folic acid further depletes the body of vitamin B_{12}, thus leading to severe neurologic damage [141, 14] Because of this hazard, vitamin B_{12} in adequate dosage should be the initial therapy whenever doubts exist about the etiology of a megaloblastic anemia [63]

Diabetic Neuropathy

Varying degrees of neural involvement were frequently found in patients with diabetes mellitus Changes in peripheral nerves as well as in the autonomic and central nervous systems may develop when the disease is inadequately controlled The pathogenesis of these neuropathies is not clear It has been suggested that they may result from the primary metabolic disturbance characteristic of the disease or from obliterative changes in the vasa nervorum, or possibly from both these factors [143] Nutritional deficiency has also been suggested as a cause [144] In this connection it is of interest to note that urinary excretion tests of vitamin B_{12} have demonstrated poor absorption of vitamin B_{12} in many diabetics,[145] and it has also been noted that some patients are more sensitive to insulin after taking vitamin B_{12} [146] These observations may account for the fact that vitamin B_{12} in large doses is often effective in treatment of the neurologic disturbances accompanying diabetes [147-149] These include polyneuritis,[147] ataxia,[144] paresthesias,[143] and nocturnal diarrhea [143, 144, 150] Impaired vibratory sense also has been reported to improve after treatment with vitamin B_1 [143]

Trigeminal Neuralgia

Vitamin B_{12} administered parenterally in large doses promptly relieves trigeminal neuralgia in a significant proportion of patients [63] Complete relief of pain has occurred,[151, 153] lasting in some cases for as long as 8 months [151] In some patients who have undergone nerve block or extirpation procedures pain relief has not occurred as promptly, but ultimate results have been equally good [151, 154] Such results suggest that treatment with vitamin B_1 is advisable in patients with trigeminal neuralgia before more drastic procedures are attempted

Herpetic Neuralgia

Large intramuscular injections of vitamin B_1 have alleviated the severe pain of herpes zoster in some cases which were resistant to

other forms of therapy [155, 156] In certain patients, pain has disappeared within 24 hours, and the lesions have cleared within 48 hours [157] In one case severe itching was alleviated by vitamin B_{12} therapy [158]

Multiple Sclerosis

Because of the efficacy of vitamin B_{12} in preventing or reversing the demyelinizing effects of pernicious anemia, treatment with massive doses of the vitamin has been tried in many cases of multiple sclerosis The results have been variable, moreover, the spontaneous remissions characteristic of the disease make the results difficult to evaluate Striking benefit was observed after very large doses of vitamin B_{12}[159] in a few patients with epileptiform seizures, and difficulties in speech, swallowing and walking In some patients definite improvement has been observed, even to the point of rehabilitation [160] In other patients the improvements were only of a subjective nature, consisting principally of increased appetite and sense of well being [160, 161]

Miscellaneous Neuropathies

Vitamin B_{12} has been used with benefit in various neuropathies which have been resistant to other therapy [162-164] The data on its use in many of these syndromes, though too scanty at present to permit definite conclusions regarding its efficacy, indicate promising areas for further investigation

Vitamin B_{12} has been used with good results by several investigators in the treatment of syndromes accompanying alcoholism Korsakoff s psychosis was extremely responsive to treatment with vitamin B_{12} in a small group of patients[160] and a few patients with delirium tremens were also successfully treated [165] Several groups of patients with alcoholic neuritis have obtained prompt relief of pain after injections of vitamin B_{12} [166-169]

Other painful neuritic syndromes have also been reported to respond to very large doses of vitamin B_{12} These include radiculitis,[159] neuritis of the cranial nerves,[159] and peripheral neuritis [159, 170] In the symptomatic treatment of peripheral neuritis it was found that only the pain which arose from pathologic involvement of the entire sensory neuron including the ganglion cells, was ameliorated by vitamin B_{12} therapy [170]

It has been reported that vitamin B_{12} in large doses (1,000 micro grams per week) is effective in relieving the neuritic pain which may

accompany arthritis,[171] particularly when the symptoms are radic in origin [17*] It has also been recommended for the treatment of nutritional deficiency or anemia which may accompany arthritis [1

Unconfirmed reports indicate the possibility of benefit from min B_{12} in other neuropathies Parenteral administration of l doses over long periods has apparently resulted in amelioratio regression of some cases of neuroblastoma in children [174] There l also been reports of prevention or alleviation of paralysis by vit B_{12} in poliomyelitis [175,176]

Liver Disorders

Numerous animal studies have indicated that vitamin B_{12} has a tective effect on the liver during certain types of dietary deficienc during intake of toxic substances Rats on an unsupplemented diet, for example, developed fatty livers which could be at least tially prevented by supplementation with methionine, choline or min B_{12} [177] Fatty livers were also prevented in alcohol intoxicated by daily doses of the vitamin [178] Protective results were even r strikingly demonstrated when two groups of rats were fed a cys free diet, with vitamin B_{12} supplementation in only one group unsupplemented group developed, within a year, severe liver char including focal necrosis, fatty metamorphosis, cirrhosis, duct hy plasia or cysts The group receiving vitamin B_{12}, on the other h showed evidence of liver disease only after two years, and even the lesions were minimal [179] That vitamin B_{12} may affect liver regen tion after injury was indicated by another study in which rats defic in riboflavin or vitamin B_{12}, or given supplements of these vitan were subjected to partial hepatectomy A higher mortality rate more limited regeneration occurred in the vitamin B_{12} deficient than in the vitamin B_{12} supplemented, riboflavin deficient, or cor groups [180]

Encouraging results have been reported with the use of liver tract[181] or vitamin B_{12} in clinical studies of liver disease The vita successfully reversed the fatal progress in all but the most advai cases of infantile biliary cirrhosis [182 183] In patients with fatty infi tion associated with diabetes or chronic alcoholism, the tender and excess volume of the liver receded rapidly following admini tion of vitamin B_{12} [167] In a preliminary investigation, vitamin B_{12} added to the conventional dietary treatment of a large grouj

patients with viral hepatitis, who were compared with two control groups. The vitamin B_{12}-treated patients regained normal appetite faster and liver size became normal more rapidly, serum bilirubin values returned to normal in 10 weeks, compared to 18 and 24 weeks in the other two groups, and the mean duration of illness was shortened by one week.[184] In a more recent study, 44 patients with viral hepatitis were given only the conventional diet, while a similar group was given, in addition, vitamin B_{12} and folic acid. The results in the two groups closely paralleled those of the earlier study.[185] Though not all investigators report success in the treatment of viral hepatitis with vitamin B_{12},[186] results such as those described suggest that vitamin B_{12} may be a useful adjunct in therapy of hepatic disorders.

Studies in patients have indicated that the metabolism of vitamin B_{12} is altered in both acute and chronic liver disease. Marked elevation of the serum vitamin B_{12} levels has been found in several studies on patients with liver damage.[187, 191] As the patients improve clinically, the serum vitamin B_{12} concentrations tend to return toward the normal range, and conversely, when patients in hepatic coma become moribund the serum vitamin B_{12} levels become more abnormal.[191] In hepatic coma levels of from 30 to 40 times normal were found.[189] In viral hepatitis the serum concentrations have ranged as high as 20 times the usual values.[190] In alcoholic cirrhosis the elevation has been 3 to 8 times normal.[188] It has been suggested that the reason for the elevated serum levels is the release of the vitamin by damaged liver cells.[190, 191] Lowered uptake by damaged liver cells, as demonstrated by studies with radioactive vitamin B_{12}[192] may also contribute to the elevated serum levels.

The binding of vitamin B_{12} by the serum in liver disease, as well as the total quantity present, varies with the nature of the disorder. It has been found that patients with acute inflammation and necrosis have high levels of the free vitamin[191, 193] while patients with chronic liver involvement have high levels of the bound vitamin.[191] It is believed that the increase in bound vitamin in such cases may be due to the production of abnormal amounts of binding substance by the damaged liver.[191] It has been postulated that much of the vitamin found in the serum in such cases may be abnormal and thus physiologically inactive and that the body tissues might be relatively unsaturated in spite of the high serum levels. Thus, a deficiency of physiologically active vitamin B_{12} might exist even in the presence of the high serum

levels This is of interest in view of the reports of benefit from vitamin B_{12} therapy in liver disease [184,185]

Since the metabolism of vitamin B_{12} is changed in liver disease, it has been suggested that such alterations might assist in evaluating the clinical status of patients [193,194] In a study of 3 normal subjects, and of 20 patients, 13 of whom had liver disorders, the normal subjects excreted between 24 and 40 micrograms of vitamin B_{12} in eight hours following a 50 microgram intramuscular load dose, but none of the patients except some who were convalescing from liver disorders excreted more than 10 micrograms In view of these findings, the authors suggest that increased vitamin B_{12} excretion after a load dose might indicate that the liver had recovered from damage and no longer needed increased amounts of vitamin B_{12} for normal metabolic activity It is also suggested that the excretion following a load dose may aid in detecting the extent of liver damage and the progress of convalescence [194] In line with this, it has been suggested[193] that the changes in serum binding capacity which characterize some types of liver disease may also help in evaluating the clinical status or in differentiating various types of liver involvement In infectious hepatitis and in homologous serum jaundice, for example, binding capacities were found to be very low during the period of hyperbilirubinemia, but rose during convalescence In cirrhosis, however, the binding capacity decreased little or not at all Moreover, the total serum levels were generally lower in cirrhosis than in hepatitis The authors suggest that these differences might be employed in conjunction with the standard tests of liver function to aid in the differential diagnosis of these liver disorders

Skin Disorders

Several investigators have reported that vitamin B_{12} given parenterally in large doses, exerts a beneficial effect on some skin disorders

Lupus Erythematosus

Investigators have reported varying results with the use of vitamin B_{12} in the treatment of lupus erythematosus In a preliminary study of 3 patients with chronic discoid and one with subacute disseminated lupus erythematosus, good response in clearing of the skin lesions was obtained with intramuscular injection of small doses, and further investigation with larger doses was recommended [195] In another study

complete clearing of the skin lesions in 3 patients who had failed to respond to other therapy was reported following 12 semi weekly injections of 1,000 micrograms of vitamin B_{12} [196]

In a study of 17 patients with chronic discoid lupus erythematosus, intramuscular injections of 1,000 micrograms three times weekly gave varying responses In one patient the lesions cleared almost completely, 2 were much improved and 2 were slightly improved In 9 patients there was no change in the lesions and in 3 the condition became worse, necessitating a change of treatment [197]

In another study it was reported that discoid as well as disseminated lesions responded even to small doses of vitamin B_{12} Healing occurred without new pigmentation, scarring, or atrophy, but atrophy already present could not be reversed [198]

Seborrheic Dermatitis

In 16 of 36 patients with seborrheic dermatitis given supplemental treatment with intramuscular vitamin B_{12} great improvement occurred, and moderate improvement was observed in 16 others The few relapses which took place responded to further therapy Other metabolic, endocrine or nutritional therapy was prescribed as needed and foci of infection were treated with sulfonamides or penicillin The investigators believed the favorable results obtained with vitamin B_{12} were based on the possible nutritional origin of this skin disorder [199]

Other Dermatoses

In a study of the use of intramuscular vitamin B_{12} in various skin disorders,[200] substantial improvement was noted in 6 out of 10 patients with atopic dermatitis, in all of 6 with chronic contact dermatitis and in 9 out of 10 with chronic urticaria In 2 patients with dermatitis herpetiformis, however vitamin B_1 was ineffective

Some patients with skin manifestations of drug reactions have benefited from the administration of vitamin B_1 Skin lesions and pruritus caused by therapeutic administration of gold salts have been alleviated or prevented by vitamin B_{12} [201] In a recent study of various dermatoses of allergic etiology it was reported that in all of 5 patients taking penicillin streptomycin injections and in all of 4 using iodine topically dermatitis healed after vitamin B_1 therapy Eight of these patients were able to continue the offending medication [02]

Several investigators have reported that skin lesions, as well as th neurologic symptoms, of patients with herpes zoster were improved by vitamin B_{12} therapy[155-157] It has been reported that vitamin B_{12} administered parenterally caused a favorable result in 31 out of 35 patients with xanthelasma[203]

In a preliminary study of the treatment of patients with long standing psoriasis, large intramuscular injections of vitamin B_{12} produced involution of lesions in 11, 75 to 80 per cent improvement in 10, gradual improvement in 6, temporary improvement in 5, and no improvement in 2 patients[204] Some confirmation of these results is provided by a report on 17 resistant cases of psoriasis in which vitamin B_{12} caused sustained improvement in 9, some improvement in 3, and no improvement in 5[205]

Endocrine Disturbances

Studies in animals have indicated that an increased vitamin intake is beneficial in certain hypohormonal or hyperhormonal states For example, supplements of vitamin B_{12} enabled rats to withstand the effects of large doses of cortisone, thyroid, thiouracil, and diethylstilbestrol, as well as the stress of adrenalectomy and thyroparathyroidectomy

The reason for such beneficial effects is not completely understood It has been suggested, however, that the improved appetite and food utilization accompanying the increased vitamin intake may have been responsible, a hypothesis which is borne out by the fact that such effects occurred only when unlimited food was available[206]

It has been postulated that during hyperhormonal states, additional vitamins are needed to detoxify or eliminate the excess hormone In hormonal deficiency states, on the other hand, the increased vitamin intake might permit metabolic reactions to proceed at an improved rate in spite of the deficiency[206]

One interesting paradox has been noted in this connection It is commonly thought that the metabolic rate determines the requirements for the B vitamins Thus, in states characterized by a reduced metabolism, the need for these vitamins should be less Nevertheless, it has been noted that in hypothyroidism and diabetes mellitus, for example, beneficial effects have followed their administration[06]

Such observations have led to speculation as to whether or not vitamin B_1 plays a part in the functioning of the endocrine system

Adrenal Function

On the basis of observations made on patients with pernicious anemia it has been suggested[207] that a deficiency of vitamin B_{12} might lead to functional impairment of the adrenal cortex or the anterior lobe of the pituitary gland, either as a result of the general debilitating effect of the deficiency or because vitamin B_{12} is actually necessary for the proper functioning of the glands If vitamin B_{12} is necessary for proper adrenal function it is possible that a metabolic antagonist to vitamin B_{12} might induce adrenocortical insufficiency Manifestations of adrenal insufficiency were reported to occur more often in pernicious anemia patients than in those with comparable degrees of anemia due to loss of blood [207] In a recent study, however,[208] no evidence was found that adrenocortical function or steroid metabolism was impaired in 8 patients with pernicious anemia in relapse

Thyroid Function

Anemia due to endocrine deficiency is most common in association with hypothyroidism Anemia also accompanies insufficiency of the anterior pituitary and of the adrenal cortex In these anemias, the bone marrow is somewhat hypoplastic Although the anemia associated with severe hypothyroidism is usually hypochromic, macrocytic anemia has also been observed and these anemias clearly represent deficiencies of iron and vitamin B_{12} superimposed on thyroid deficiency Thyroid deficiency alone may limit the rate of hemopoiesis The treatment of the mild anemia of endocrine disorders, however, is largely incidental to the management of the conditions themselves

In humans it has been noted that patients suffering from pernicious anemia complicated by severe hypothyroidism may require higher dosages of thyroid extract when they are receiving vitamin B_{12} In some such cases the dosage had to be doubled to effect a normal metabolic rate [209] Because of this observation it has been postulated that vitamin B_{12} has antithyrotoxic effects and therefore might be a useful adjunct in the treatment of thyrotoxicosis [209] However, further investigation is necessary to test the validity of this idea

Diabetic Retinopathy

The ocular complications of diabetes have a definite relationship to the duration of the disease and to its control While the pathogen esis of these lesions is not yet understood, recent studies on vitamin

B_{12} and diabetic retinopathy have thrown some light on the etiology of this complication [210] Some association has been indicated between adrenocortical function, vitamin B_{12}, and diabetic retinopathy Decreased adrenocortical function, as measured by responsiveness to corticotropin, was found in diabetic patients without retinopathy, while those with retinopathy had normal or increased function [211] A relationship between retinopathy and Kimmelstiel Wilson lesions has been indicated by studies which showed that at autopsy 80 to 90 per cent of diabetic patients with nephropathy also had retinopathy, and about 50 per cent of those with symptoms of retinopathy proved at autopsy to have kidney lesions [212]

Since study in humans showed that the urinary excretion of vitamin B_{12} given intramuscularly was much less from diabetic patients than it was from normal subjects,[213] it was thought that a relationship might exist between the amount of vitamin B_{12} excreted in the urine of diabetic patients and the presence or absence of retinopathy To test this hypothesis, 50 micrograms of vitamin B_{12} was given to 35 diabetic patients, 22 with and 13 without retinopathy, and to 6 healthy persons Diabetic patients with retinopathy excreted an average of approximately 19 micrograms of vitamin B_{12} in eight hours, those without retinopathy an average of only 4 2 micrograms, and normal control subjects an average of 9 6 micrograms These differences between patients with and without retinopathy were statistically significant, but the authors were unable to explain their meaning, furthermore no correlation was found between the severity of the retinopathy and the vitamin B_{12} excretion [213] Depression of adrenocortical function in diabetic patients with retinopathy by administration of testosterone caused a decrease in their excretion of vitamin B_{12} [145] As a result of such studies it has been suggested that excessive adrenocortical function and a deficiency of vitamin B_{12} may be involved in Kimmelstiel-Wilson and retinopathic lesions [145]

Of interest in connection with the relationship of vitamin B_{12} to complications of diabetes, such as diabetic retinopathy, are studies in animals indicating that vitamin B_{12} is involved in carbohydrate metabolism In rats, vitamin B_{12} deficiency causes hyperglycemia which is corrected by administration of the vitamin Moreover, cortisone induced hyperglycemia is accompanied by destruction of vitamin B_{12} binding substances in muscle, and an elevated vitamin B_{12} serum level Whether this has any connection with the apparent adrenal hyper

activity and increased excretion of the vitamin found in patients with retinopathy has not yet been determined [214]

Clinical Indications for Dietary Supplementation

Nutritional disorders may result from any of the following factors poor dietary intake, poor absorption, metabolic disturbances leading to nausea, vomiting diarrhea, and increased metabolism, and stress conditions such as severe or prolonged infection, injury, or surgery, particularly surgery of the gastrointestinal tract It is well established that vitamin supplementation is beneficial in such conditions and that vitamin B_{12} plays an important role in such supplementation [215]

Retarded Growth

Since large scale depletion studies are usually not conducted in human beings, the most significant findings are evident in infants or children who are deprived of animal protein for prolonged periods because of inadequate diet or who are unable to assimilate vitamin B_{12} because of illness

Early controlled studies on oral administration of vitamin B_{12} to undernourished children with growth failure showed that statistically significant growth increases occurred in the treated children [216 217] Later studies on a large group of underweight Italian children who were eating diets low in animal protein also showed that those receiving supplements of vitamin B_{12} gained more weight than the controls [218] These findings are supported by other controlled studies [219-221] Uncontrolled studies in malnourished children have also indicated beneficial effects, but results in these groups are, of course, difficult to evaluate [222 223] Although these studies in general show a growth effect in malnourished children with retarded growth, no beneficial effect was demonstrated in other controlled studies [224-227]

When normally nourished but chronically ill children received vitamin supplements including vitamin B_{12} their rate of growth was significantly greater than that of the controls [118 119] Beneficial effects including increases in weight and improved blood protein levels, were also observed in acutely ill children with respiratory and cardiovascular disease following administration of vitamin B_{12} and liver extract [230] These results on sick children indicate that the need for vitamin B_{12}, like the need for other vitamins, may be increased during illness

Because many studies indicate increased growth in children fol

lowing vitamin B_{12} therapy, it has been suggested that the vitamin may participate in some fundamental metabolic growth regulating mechanism[221] It must be emphasized, however, that most of these studies showed a beneficial effect of vitamin B_{12} on growth only in poorly nourished children Further extensive investigation is necessary before definite conclusions can be reached

Vitamin B_{12} has produced beneficial effects on anorexia in children Increased appetite has been reported in two groups of healthy, but anorectic, children given supplemental vitamin B_{12} [231 232] In a study of underweight children, 114 of 145 showed a marked increase in appetite, which was reflected in an increase in rate of weight gain[233]

Pregnancy

Several studies indicate that an increased need for vitamin B_{12} may exist during pregnancy, possibly because large amounts of the vitamin are needed for growth and blood formation in the fetus A comparative study in pregnant and nonpregnant women indicates that the absorption of vitamin B_{12} from the intestine may be increased during pregnancy[234] That this increased absorption may be a mechanism of compensation for the depletion of the mother's vitamin B_{12} stores is suggested by several studies which show that maternal vitamin B_{12} serum levels progressively decrease during pregnancy[133 234-236] Assays of the serum obtained from 502 women at various stages of pregnancy showed an unmistakable tendency for a decrease of the vitamin B_{12} serum level with the progress of pregnancy Even during the first trimester the average value was below that previously established for normal males and nonpregnant females[235] A study of pregnant women during the second and third trimester indicates that the serum levels of vitamin B_{12} may decrease to levels comparable to those found in pernicious anemia[236] A study made during the third trimester showed that normal or low vitamin B_{12} serum levels progressively decreased until after delivery, when they rose again[133]

In another study vitamin B_{12} serum levels were determined in 25 mothers and infants at the time of delivery[237] It was found that the vitamin B_{12} serum level of the maternal blood was considerably lower than that of nonpregnant women of comparable age It was also lower than the vitamin B_{12} level in the fetal cord blood These results were confirmed by a study of 96 paired serum samples taken from mothers and infants at delivery which showed that the average serum vitamin

B_{12} concentration in the infant was about twice that in the mother. In some cases the infant's vitamin B_{12} serum concentration was six times that of the mother.[238] These studies suggest the possible need for supplementary vitamin B_{12} during pregnancy even in the absence of frank macrocytic anemia.

Several animal studies provide evidence that vitamin B_{12} is necessary for the proper growth and development of the fetus. Deprivation of vitamin B_{12} in female rats, for example, throughout the period of mating and gestation, resulted in weak, defective, underweight offspring. Developmental defects were noted in the heart and kidneys, and in the spleen the reticular cells were large, densely chromatic and pyknotic. Vascular blocks with passive congestion, especially in the liver, were observed.[239] In a study on a different strain of rats, the congenital anomalies resulting from vitamin B_{12} deficiency took the form of hydrocephalus or defects in eye and bone development.[240]

While it is difficult to apply the results of these studies to human beings, they suggest the need for further studies on the effect of vitamin B_{12} on the fetus, particularly since it has been established that maternal vitamin B_{12} serum levels are frequently lowered in pregnancy.

Clinical Indications in Geriatric Patients

Vitamin deficiencies may occur in the elderly patient when, because of poor dentition, poverty, indifference or chronic gastritis, an inadequate diet is taken.[241] Even when their diet is normal, however, elderly people frequently have special nutritional problems because of the physiologic changes which accompany aging.[241] Improvement in the status of geriatric patients following the administration of vitamin B_{12} alone or in combination with other vitamins has been reported.[242, 243]

One physiologic change observed in older persons is a decreased capacity of the gastric juice to bind vitamin B_{12}.[244, 245] Achlorhydria and hypochlorhydria are also found in the aged and it is probable that vitamin B_{12} deficiency due to poor absorption may occur with some frequency in the aged.[246] This is consistent with the observation that older people absorb orally administered vitamin B_{12} less readily than do younger persons. In a recent study, a group of persons between 20 and 40 years of age all showed a significant increase in the serum vitamin B_{12} level after a large oral dose, while only 6 out of 18 in the group between 60 and 90 years of age showed a comparable increase.[244]

Decreased absorption of orally administered vitamin B_{12} in older

persons results in lower blood levels of the vitamin[247] and probably in lower tissue levels [244] Assays of serum samples from randomly selected populations of different age groups show that the vitamin B_{12} serum content decreases with advancing age [248] In a group of old individuals on a satisfactory diet containing adequate vitamin B_1 , the mean serum vitamin B_{12} level was significantly lower than the mean serum level found in a group of younger individuals on a normal diet [248] In a study of five groups of apparently healthy subjects, composed of a total of 528 individuals ranging in age from 12 to 94, it was found that the mean serum vitamin B_{12} levels in each group showed an almost uniform gradual decline with advancing age Low serum vitamin B_{12} levels were found more frequently in persons over 50 years old than in younger persons [249] That the tissue levels as well as the blood levels of vitamin B_{12} in older persons are decreased is indicated by studies which show that more injected vitamin B_{12} is retained by older than by younger persons, indicating less vitamin B_{12} saturation in the tissues of the former [244]

In a discussion of the nutritional rehabilitation of the elderly patient undergoing surgery, one investigator has noted that subclinical vitamin deficiencies are common among this group because of anorexia, lack of balanced diet, infection, intestinal disorders and long illnesses Therefore vitamin B_{12} was suggested as an adjunct to therapy in the elderly patient undergoing operation or any other severe stress [250]

Stress

The findings of several investigators have suggested that the need for vitamin B_{12} during periods of stress may be increased beyond the amount supplied by the diet For example, increased vigor and sense of well-being have frequently been observed in patients with a variety of illnesses who have been treated with vitamin B_{12}, even when no direct effect upon the disease was apparent [197, 251-253] In children, the most striking growth increments have followed vitamin B_{12} therapy in those who were undersized because of previous illness or inadequate nutrition [216, 217, 228, 230]

In line with these observations, the suggestion has been made that patients with illness or trauma, as well as those depleted nutritionally, be given supplemental vitamin B_{12} [215] It has been pointed out in this connection that during severe illness, following burns, after radiation injury, or in certain pathologic states, hemopoiesis may be depressed

and enzyme activity may be disturbed Since vitamin B_{12} is involved in normal hemopoiesis and probably in some enzymatic processes, its regular administration, along with folic acid, has been recommended as part of the dietary supplements used in hospitals when the diet intake is limited [254]

REFERENCES

1 Shorb M S Activity of vitamin B_{12} for the growth of *Lactobacillus lactis* *Science* 107 397–398, April 16 1948

2 Hoffmann, C E, Stokstad E L R, Franklin, A L and Jukes, T H Response of *Lactobacillus leichmannii* 313 to the antipernicious anemia factor *J Biol Chem* 176 1465–1466, Dec 1948 (in Letters to the Editors)

3 Davis B D and Mingioli E S Mutants of *Escherichia coli* requiring methionine or vitamin B_{12} *J Bact* 60 17–28 July 1950

4 Hutner S H Provasoli, L, Stokstad E L R, Hoffmann C E, Belt M, Franklin A L and Jukes T H Assay of anti pernicious anemia factor with *Euglena* *Proc Soc Exper Biol & Med* 70 118–120 Jan 1949

5 Hutner S H Provasoli L and Filfus J Nutrition of some phagotrophic fresh water chrysomonads *Ann New York Acad Sc* 56 852–862 Oct 14 1953

6 Rickes E L Brink, N G Koniuszy F R, Wood T R and Folkers K Comparative data on vitamin B_{12} from liver and from a new source *Streptomyces griseus* *Science* 108 634–635 Dec 3 1948 (in Comments and Communications)

7 Hendlin, D and Ruger M L The effect of cobalt on the microbial synthesis of LLD active substances *Science* 111 541–542 May 19 1950

8 Hollenbeck, C M Monahan R Benson W L and Mahoney J F Some preliminary considerations of vitamin B_{12} fortification of foods *Tr Am A Cereal Chemists* 13 233–240 Oct 1955

9 Zucker T F and Zucker L M 'Animal Protein Factor" and Vitamin B_{12} in the Nutrition of Animals in Vitamins and Hormones New York Academic Press Inc 1950 vol 8 pp 1–54

10 Ott W H Rickes E L and Wood T R Activity of crystalline vitamin B_{12} for chick growth *J Biol Chem* 174 1047–1048 July 1948 (in Letters to the Editors)

11 Emerson G A Wurtz E and Zanetti M E Vitamin B_{12}—a growth factor for young rats *Federation Proc* 8 381–382 March 1949

12 Bosshardt D K Paul, W J O'Doherty K, Huff, J W and Barnes R H Mouse growth assay procedures for the animal protein factor' *J Nutrition* 37 21–35, Jan 1949

13 Hogan, A G and Anderson, G C Vitamin B_{12} in swine nutrition, *Federation Proc* 8 385–386 March 1949

14 Alexander, W F and Backlar, B Nucleic acid changes in rat nerve tissue after parenteral administration of vitamin B_{12} *Proc Soc Exper Biol & Med* 78 181 184 Oct 1951

15 Rose I A and Schweigert, B S Effect of vitamin B_{12} on nucleic acid metabolism of the rat *Proc Soc Exper Biol & Med* 79 541–544 March 1952

16 Glazer, H S, Mueller J F Jarrold T, Sakurai, K Will, J J and Vilter R W The effect of vitamin B_{12} and folic acid on nucleic acid composition of the bone marrow of patients with megaloblastic anemia *J Lab & Clin Med* 43 905–913 June 1954

17 Shive, W The Functions of B Vitamins in the Biosynthesis of Purines and Pyrimidines in Vitamins and Hormones, New York, Academic Press Inc 1951, vol 9 pp 75–130

18 Bennett M A Utilization of homocystine for growth in presence of vitamin B_{12} and folic acid *J Biol Chem* 187 751–756 Dec 1950

19 Schaefer, A E Salmon, W D and Strength D R Role of vitamin B_{12} and methyl donors in lipotropism and transmethylation in the rat and chick *Federation Proc* 9 369–370 March 1950

20 Arnstein, H R V and Neuberger A The effect of cobalamin on the quantitative utilization of serine, glycine and formate for the synthesis of choline and methyl groups of methionine *Biochem J* 55 259–271 Feb 1953

21 Chang I and Johnson B C The effect of vitamin B_{12} on some aspects of glycine metabolism *Arch Biochem & Biophys* 55 151–156 March 1955

22 Johnson, B C Firth J and Mistry S P Vitamin B_{12} and choline synthesis from glycine *in vivo* *Arch Biochem & Biophys* 54 467–473 Feb 1955

23 Young, R J Norris L. C. and Heuser G F The utilization by vitamin B_{12}-deficient chicks of monomethylaminoethanol homocystine and betaine as precursors of choline and methionine *J Nutrition* 53 233–248 June 1954

24 Gillis M B and Norris L. C. Methylation of homocystine by chicks deficient in vitamin B_{12} *Proc Soc Exper Biol & Med* 77 13–15 May 1951

25 Firth J Mistry S P James M F and Johnson, B C. Vitamin B_{12} and transmethylation in the baby pig *Proc Soc Exper Biol & Med* 85 307–309 Feb 1954

26 Ling C. T and Chow B F The effect of vitamin B_{12} on the levels of soluble sulfhydryl compounds in blood *J Biol Chem* 202 445–456 May 1953

27 Register U D Effect of vitamin B_{12} on liver and blood non protein sulfhydryl compounds *J Biol Chem* 206 705–709 Feb 1954

28 Boxer G E. Ott, W H and Shonk, C. E. Influence of vitamin B_{12} on the coenzyme A content of the liver of chicks *Arch Biochem & Biophys* 47 474–475 Dec 1953 (in Letters to the Editors)

29 Boxer G E. Shonk C. E Gilfillan E. W Emerson G A and Oginsky E. L. Changes in coenzyme A concentration during vitamin B_{12} deficiency *Arch Biochem & Biophys* 59 24–32 Nov 1955

30 Wong, W T and Schweigert, B S Role of vitamin B_{12} in nucleic acid metabolism II Liver coenzyme A levels in the rat *Arch Biochem & Biophys* 60 126–129 Jan 1956

31 Dietrich, L.S Monson W.J. and Elvehjem C A. Observations on a relationship between vitamin B_{12} folic acid and the citrovorum factor *Proc Soc Exper Biol & Med* 77 93–96 May 1951

32 Doctor V M Elam, J F Sparks P Lyman, C. M and Couch, J R Studies on the conversion of folic acid to citrovorum factor by avian liver homogenate *Arch Biochem & Biophys* 48 249–255 Feb 1954

33 Spray G H and Witts L J Conversion of folic acid to the *citrovorum factor* in health and pernicious anaemia *Brit M J* 2 62–63 July 12 1952

34 Doctor V M Couch, J R and Trunnell J B Factors influencing conversion of folic acid to citrovorum factor by chick liver fractions *Proc Soc Exper Biol & Med* 87 228–231 Oct 1954

35 Rupp J Paschkis K E and Cantarow A Influence of vitamin B_{12} and liver extract on nitrogen balance of normal and hyperthyroid rats, *Proc Soc Exper Biol & Med* 76 432–435 March 1951

36 Catron D V Richardson D Underkofler L. A Maddock H M and Friedland W C. Vitamin B_{12} requirement of weanling pigs II Performance on low levels of vitamin B_{12} and requirement for optimum growth *J Nutrition* 47 461–468 July 1952

37 Hsu J M Stern J R and McGinnis J Effect of vitamin B_{12} deficiency on chick plasma proteins *Arch Biochem & Biophys* 42 54–60 Jan 1953

38 Black A and Bratzler J W The effects of a vitamin B_{12} supplement, vitamin B_{12} and streptomycin on the metabolism of the rat, *J Nutrition* 47 159–173 June 1952

39 Meites J and Feng Y S L. Prevention by vitamin B_{12} of protein catabolic action of cortisone, *Proc Soc Exper Biol & Med* 85 341–344 Feb 1954

40 Rupp J and Paschkis K E Lack of influence of vitamin B_{12} on protein catabolic action of cortisone *Proc Soc Exper Biol & Med* 82 65–66 Jan 1953

41 Knoebel L. K and Black A The influence of vitamin B_{12} and antibiotics on protein and energy utilization in a low protein diet *J Nutrition* 48 477–487, Dec 1952

42 Zöllner N and Vogtmann, W The effect of vitamin B_{12} on the conversion of fructose in the rat liver [in German] *Klin Wchnschr* 30 761–762 Aug 15, 1952

43 Collins R A Schreiber M, Elvehjem C A and Hart, E B Vitamin B_{12} and folic acid in milk diets *J Nutrition* 49 485–494 March 1953

44 Ling C T and Chow B F The influence of vitamin B_{12} on carbohydrate and lipide metabolism *J Biol Chem* 206 797–805 Feb 1954

45 Hsu P T and Combs G F Effect of vitamin B_{12} and amino acid imbalances on growth and levels of certain blood constituents in the chick *J Nutrition* 47 73–91 May 1952

46 Spivey M R Briggs G M and Ortiz, L O Effect of diets high in fat or protein on vitamin B_{12} deficiency in non depleted chick *Proc Soc Exper Biol & Med* 85 451–453 March 1954

47 Fatterpaker, P Marfatia U and Screenivasan, A. Protective action of vitamin B_{12} in the hyperthyroid rat, *Nature* 176 165 July 23 1955

48 Meites J Beneficial effects of vitamin B_{12} and Aureomycin in rats given large doses of cortisone *Proc Soc Exper Biol & Med* 81 307–311, Oct 1952

49 Castle W B Development of knowledge concerning the gastric intrinsic factor and its relation to pernicious anemia *New England J Med* 249 603–614 Oct 8 1953 (in Medical Progress)

50 Gardner F H Harris J W Schilling R F and Castle W B Observations on the etiologic relationship of achylia gastrica to pernicious anemia XI Hematopoietic activity in pernicious anemia of a beef muscle extract containing food (extrinsic) factor upon intravenous injection without contact with gastric (intrinsic) factor *J Lab & Clin Med* 34 1502–1511 Nov 1949

51 Berk L Castle W B, Welch A D Heinle R W Anker R and Epstein M Observations on the etiologic relationship of achylia gastrica to pernicious anemia X Activity of vitamin B_{12} as food (extrinsic) factor, *New England J Med* 239 911–913 Dec 9 1948

52 Glass G B J Boyd L J Rubinstein, M A and Svigals C S Relationship of glandular mucoprotein from human gastric juice to Castle s intrinsic antianemic factor *Science* 115 101–108 Feb 1 1952

53 Williams W L Ellenbogen, L and Esposito R G Preparation of highly purified intrinsic factor *Proc Soc Exper Biol & Med* 87 400–405 Nov 1954

54 Latner A L Merrills R J and Raine L C D P Isolation of Castle s intrinsic factor *Lancet* 1 497–498 March 6 1954 (in Preliminary Communications)

55 Latner A L, Merrills R J and Raine L C D P Further observations on the isolation and chemical composition of Castle s intrinsic factor *Biochem J* 57 xix, July 1954 (in Soc Proc.)

56 Halsted J A, Gasster, M and Drenick, E J Absorption of radioactive vitamin B_{12} after total gastrectomy Relation to macrocytic anemia and to the site of origin of Castle s intrinsic factor *New England J Med* 251 161–168 July 29 1954

57 Callender S T and Lajtha L G On the nature of Castle s hemopoietic factor *Blood* 6 1234–1239 Dec 1951

58 Rosenblum C, Woodbury, D T Gilfillan E W and Emerson, G A Influence of intrinsic factor upon utilization of orally administered vitamin B_{12} *Federation Proc* 13 475 March 1954

59 Nieweg H O Arends A Mandema, E and Castle W B Enhanced absorption of vitamin B_{12} in gastrectomized rat by rat intrinsic factor *Proc Soc Exper Biol & Med* 91 328–332 Feb 1956

60 Ungley C C The Chemotherapeutic Action of Vitamin B_{12} in Vitamins and Hormones New York, Academic Press Inc, 1955 vol 13 pp 137–211

61 Baker S J and Mollin, D L The relationship between intrinsic factor and the intestinal absorption of vitamin B_{12} *Brit J Haemat* 1 46–51 Jan 1955

62 Ross G I M, Mollin D L Cox, E V and Ungley, C C Hematologic responses and concentration of vitamin B_{12} in serum and urine following oral administration of vitamin B_{12} without intrinsic factor *Blood* 9 473–488 May 1954

63 Unglaub W G and Goldsmith, G A Folic acid and vitamin B_{12} in medical practice (report to Council on Foods and Nutrition) *J.A M.A* 161 623–627 June 16 1956

64 Callender S T and Evans J R Observations on the relationship of intrinsic factor to the absorption of labelled vitamin B_{12} from the intestine *Clin Sc* 14 387–393 Aug 1955

65 McIntyre P A Sachs M V Krevans J R and Conley C L Pathogenesis and treatment of macrocytic anemia information obtained with radioactive vitamin B_{12} *A M.A Arch Int Med* 98 541–549 Nov 1956

66 Monto R W and Rebuck J W Nasal instillation and inhalation of crystalline vitamin B_{12} in pernicious anemia *A M.A Arch Int Med* 93.219–230, Feb 1954

67 Israëls, M C G and Shubert, S The treatment of pernicious anaemia by insufflation of vitamin B_{12} *Lancet* 1.341–343 Feb 13 1954

68 Monto R W and Howell J T Urinary vitamin B_{12} activity following intranasal administration, *J Lab & Clin Med* 45 474–477 March 1955

69 Pollycove M and Apt, L Absorption, elimination and excretion of orally administered vitamin B_{12} in normal subjects and in patients with pernicious anemia *New England J Med* 255.207–212, Aug 2, 1956

70 Krevans J R Conley C L and Barrows C R Jr Observations on the absorption and excretion of vitamin B_{12} *Bull Johns Hopkins Hosp* 88 568–569 June 1951 (in Soc Proc)

71 Heinle R W Welch, A D Scharf V Meacham G C and Prusoff W H Studies of excretion (and absorption) of Co^{60} labeled vitamin B_{12} in pernicious anemia *Tr A Am Physicians* 65 214–222 1952

72 Schilling, R F Intrinsic factor studies II The effect of gastric juice on the urinary excretion of radioactivity after the oral administration of radioactive vitamin B_{12} *J Lab & Clin Med* 42 860 866 Dec 1953

73 Rath C E McCurdy P R and Duffy B J Jr Effect of renal disease on the Schilling test, *New England J Med* 256 111–114 Jan 17 1957

74 Rosenblum C Chow B F Condon, G P and Yamamoto R S Oral versus parenteral administration of Co^{60} labeled vitamin B_{12} to rats *J Biol Chem* 198 915–928 Oct 1952

75 Pitney W R Beard M F and Van Loon, E J Observations on the bound form of vitamin B_{12} in human serum, *J Biol Chem* 207 143–152, March 1954

76 Unglaub W G Rosenthal H L and Goldsmith G A Studies of vitamin B_{12} in serum and urine following oral and parenteral administration, *J Lab & Clin Med* 43 143–156 Jan 1954

77 Winter C A and Mushett C W Absence of toxic effects from single injections of crystalline vitamin B_{12} *J Am Pharm A* (Scient Ed) 39 360–361 June 1950

78 Goldblatt S On the intravenous administration of cyanocobalamin *Am J Clin Nutrition* 3 129–131 March–April 1955

79 Conley C L Krevans J R Chow B F Barrows C and Lang C A Observations on the absorption utilization and excretion of vitamin B_{12} *J Lab & Clin Med* 38 84–94 July 1951

80 Conley C L and Krevans J R New developments in the diagnosis and treatment of pernicious anemia *Ann Int Med* 43 758–766 Oct 1955

81 Bedford, P D Side-effects of a preparation of vitamin B_{12} *Brit M J* 1 690–691 March 29 1952

82 Noren, B Allergic reactions in parenteral liver therapy and vitamin B_{12} *Acta med Scandinav* 137 48–65 1950

83 Castle W B Present status of the etiology of pernicious anemia *Ann Int Med* 34 1093–1106 May 1951

84 Campbell D C, Hall B E and Morgan E H Oral administration of vitamin B_{12} in pernicious anemia II Studies on the nature and source of intrinsic factor *J Lab & Clin Med* 34 1590 Nov 1949 (In Proc Soc)

85 Vilter R W Treatment of macrocytic anemias *A M A Arch Int Med* 95 482–492, March 1955 (in Treatment in Internal Medicine)

86 Campbell D C Hall B E and Morgan E H The treatment of pernicious anemia and other macrocytic anemias with vitamin B_{12} *M Clin North America* 34 1197–1204 July 1950

87 Conley C L Green, T W Hartmann R C and Krevans J R Prolonged treatment of pernicious anemia with vitamin B_{12} *Am J Med* 13 284–293 Sept 1952

88 Schwartz S O Friedman I A and Gant H L Long term evaluation of vitamin B_{12} in treatment of pernicious anemia I Incidental report on use of combined oral therapy with vitamin B_{12} and folic acid *J A M A* 157.229–231 Jan 15 1955

89 Schwartz M Lous P and Meulengracht E Reduced effect of heterologous intrinsic factor after prolonged oral treatment in pernicious anaemia *Lancet* 1 751–753 April 13 1957

90 Hall B E Morgan E H and Campbell D C Studies on the nature of the intrinsic factor of Castle *Brit M J* 2 585–589, Sept 9 1950

91 Hall B E Morgan E H and Campbell, D C Oral administration of vitamin B_{12} in pernicious anemia I Presence of intrinsic factor in Berkefeld filtered pooled human gastric juice preliminary report *Proc Staff Meet Mayo Clin* 24 99–107 Feb 16 1949

92 Reisner, E H, Jr, Weiner L Schittone M T and Henck, E A Oral treatment of pernicious anemia with vitamin B_{12} without intrinsic factor, *New England J Med* 253 502–506 Sept 22 1955

93 Unglaub, W G and Goldsmith G A. Oral vitamin B_{12} in the treatment of macrocytic anemias *South M J* 48 261–269 March 1955

94 MacDonald R M Ingelfinger F J and Belding H W Late effects of total gastrectomy in man *New England J Med* 237 887–896, Dec 11 1947

95 Blake J and Rechnitzer P A The hematological and nutritional effects of gastric operations *Quart J Med* 22 419–437 Oct 1953

96 MacLean, L D and Sundberg R D Incidence of megaloblastic anemia after total gastrectomy *New England J Med* 254 885–893, May 10, 1956

97 Hitchcock C R MacLean, L D and Sullivan, W A The secretory and clinical aspects of achlorhydria and gastric atrophy as precursors of gastric cancer, *J Nat Cancer Inst* 18 795–811 June 1957

98 Pitney, W R and Beard M F Vitamin B_{12} deficiency following total gastrectomy *A M A Arch Int Med* 95 591–593, April 1955

99 Swendseid M E Halsted, J A Drenick, E and Gasster, M Absorption of vitamin B_{12} as measured by fecal excretion of cobalt[60] labelled vitamin B_{12} in relation to Castle s intrinsic factor Western Society for Clinical Research Seventh Annual Meeting Jan 29–30 1954, p 30

100 Harvey J C The vitamin B_{12} deficiency state engendered by total gastrectomy *Surgery* 40 977–989 Nov 1956 (in Recent Advances in Surgery)

101 Paulson M and Harvey J C Hematological alterations after total gastrectomy evolutionary sequences over a decade, *J A M A* 156 1556–1560 Dec 25 1954

102 Bastrup Madsen P Non anaemic neuropathy caused by deficiency of the anti pernicious anaemia principle following subtotal gastrectomy *Acta med Scandinav* 147 399–404 1954

103 Halsted J A Megaloblastic anemia associated with surgically produced gastrointestinal abnormalities *California Med* 83 212–217, Sept 1955

104 Brintnall E S Daum K Hickey R C Tidrick R T and Wickstrom A P Total gastrectomy an evaluation *J Internat Coll Surgeons* 25 409–420 April 1956

105 Goldhamer S M Pernicious anaemia syndrome in gastrectomized patients *Surg Gynec & Obst* 57 257–260 Aug 1933

106 Gordon N S and Japa J Macrocytic anaemia following gastro-enterostomy *Brit M J* 2 769–770 Nov 29, 1941

107 Badenoch, J., Evans J R Richards, W C D and Witts L J Megaloblastic anemia following partial gastrectomy and gastro-enterostomy *Brit J Haemat* 1 339–344 Oct 1955 (abstr *J A M A* 160 321–322 Jan 28 1956)

108 Halsted J A Lewis P M and Gasster M Absorption of radioactive vitamin B_{12} in the syndrome of megaloblastic anemia associated with intestinal stricture or anastomosis *Am J Med* 20 42–52 Jan 1956

109 Doscherholmen A and Hagen, P S Absorption of Co^{60} labeled vitamin B_{12} in intestinal blind loop megaloblastic anemia *J Lab & Clin Med* 44 790 Nov 1954 (in Soc Proc)

110 Pearson R D Macrocytic anemia associated with intestinal strictures and anastomoses report of two cases *Ann Int Med* 40 600–610 March 1954

111 Ungley C C The pathogenesis of megaloblastic anaemias and the value of vitamin B_{12} *Brit J Nutrition* 6 299–315 1952

112 von Bonsdorff B and Gordin, R Oral administration of vitamin B_{12} in pernicious tapeworm anemia *Diphyllobothrium latum* and pernicious anemia *Acta med Scandinav* 140 112–122, supp 259 1951

113 Bjorkenheim, G Influence of folic acid on the nervous system in pernicious anemia Experiences relating to pernicious tapeworm anemia *Acta med Scandinav* 145 406–409 1953

114 Wokes F Badenoch J and Sinclair H M Human dietary deficiency of vitamin B_{12} *Am J Clin Nutrition* 3 375–382, Sept–Oct 1955

115 Wokes F Nutrition and vitamin B_{12} *Lancet* 2 1343 Dec 24 1955 (in Letters to the Editor)

116 Wokes F Vegetarian diet, *Brit M J* 1 683 March 24 1956 (in Soc Proc)

117 Wokes F and Picard C W The role of vitamin B_{12} in human nutrition *Am J Nutrition* 3 383–390 Sept–Oct 1955

118 Das Gupta C R Chatterjea J B Ghosh S K and Banerjee D K Vitamin B_{12} concentration of serum in nutritional macrocytic anaemia *Bull Calcutta Sch Trop Med* 3 101 1955 (abstr *Blood* 11 688–689 July 1956)

119 Foy H Kondi A and Manson Bahr P E C Penicillin in megaloblastic anaemias of Africans Effect on serum vitamin B_{12} levels and absorption of radioactive B_{12} *Lancet* 2 693–699 Oct 1 1955

120 Vilter R W Essential nutrients in the management of hemopoietic disorders of human beings *Am J Clin Nutrition* 3 72–74 Jan Feb 1955

121 Edgren G and Segerdahl E Megalocytic anemia in infants *Acta med Scandinav* 134 235–243 1949

122 Woodruff C W Ripy H W Peterson, J C and Darby W J Variable response to vitamin B_{12} of megaloblastic anemia of infancy *Pediatrics* 4 723–729 Dec 1949

123 May C D Nelson, E N Lowe C U and Salmon, R J Pathogenesis of megaloblastic anemia in infancy *A M A Am J Dis Child* 80 191–206 Aug 1950

124 Sturgeon, P and Carpenter G Megaloblastic anemia of infancy Response to vitamin B_{12} *Blood* 5 458–467 May 1950

125 Luhby A L and Wheeler W E Megaloblastic anemia of infancy II—Failure of response to vitamin B_{12} and the metabolic role of folic acid and vitamin C *Health Center J* (Ohio State University) 3 1–20 Dec 1949

126 Bethell F H Treatment of anemia *Wisconsin M J* 51 1082–1084 Nov 1952

127 Panels in therapy XI The treatment of sprue *Blood* 11 570–576 June 1956

128 Di Riamondo F La Pesa M and Conese G Steatorrhea from antibiotics *Gior mal infet e parassit* 5 389 Nov–Dec. 1953 (abstr *A M A Am J Dis Child* 89 111 Jan 1955)

129 Diéz Rivas F Hernández Morales, F and Meyer L M The oral use of combined vitamin B_{12} and folic acid in tropical sprue *Ann Int Med* 36 1076–1085 April 1952

130 Diéz Rivas, F Suárez, R M Hernández Morales F H and Santiago E. P The oral administration of vitamin B_{12} in tropical sprue *Ann Int Med* 36 583–591 Feb 1952 (Part 2)

131 Spies, T D Garcia Lopez G Milanes F Lopez Toca R L and Aramburu T A note on the oral versus parenteral administration of vitamin B_{12} *South M J* 42 528–531 June 1949

132 Goldsmith G A Effects of vitamin B_{12} given orally and parenterally and of a concentration of intrinsic factor in macrocytic anemias *Am J Med* 9 399 Sept 1950

133 Izak G Rachmilewitz M Stein Y Berkovici B Sadovsky A Aronovitch Y and Grossowicz, N Vitamin B_{12} and iron deficiencies in anemia of pregnancy and puerperium *A M A Arch Int Med* 99 346–355 March 1957

134 Berry C G Anaemia of pregnancy in Africans of Lagos *Brit M J* 2 819–823 Oct 1 1955

135 Moore H C Lillie E H and Gatenby P B B The response of megaloblastic anaemia of pregnancy to vitamin B_{12}, *Irish J M Sc* 351 106 March 1955 (abstr *Am J Obst & Gynec* 71 1163 May 1956)

136 Holly R G Megaloblastic anemia in pregnancy Remission following combined therapy with ascorbic acid and vitamin B_{12} *Proc Soc Exper Biol & Med* 78 238–241 Oct 1951

137 Lowenstein L Pick C and Philpot N Megaloblastic anemia of pregnancy and puerperium *Am J Obst & Gynec* 70 1309–1337 Dec 1955

138 Alexander W F Neuropathology in vitamin B_{12} deficiency Nutrition Symposium Series No 7 National Vitamin Foundation, Inc New York 1953 pp 47–65

139 Wintrobe, M M Clinical Hematology 4th ed Philadelphia Lea and Febiger 1956 p 475

140 Holmes J M Vitamin B_{12} deficiency, *Brit M J* 2 228, July 28 1956 (in Soc Proc)

141 Darby W J Folic acid and citrovorum factor in human nutrition Nutrition Symposium Series No 7, National Vitamin Foundation Inc New York, 1953 pp 85–99

142 Vilter R W Horrigan D Mueller, J F Jarrold T, Vilter C F Hawkins V and Seaman, A Studies on the relationships of vitamin B_{12} folic acid thymine uracil, and methyl group donors in persons with pernicious anemia and related megaloblastic anemias *Blood* 5 695–717, Aug 1950

143 Sancetta S M Ayres, P R and Scott R W The use of vitamin B_{12} in the management of the neurologic manifestation of diabetes mellitus with notes on the administration of massive doses *Ann Int Med* 35 1028–1048, Nov 1951

144 Beidleman, B Clinical vitamin deficiencies in patients with diabetes mellitus, *Am J Clin Nutrition* 1 119–123 Jan 1953

145 Becker B, Allen, R, Winter F C Maengwyn Davies, G and Friedenwald J S The role of the adrenal cortex and vitamin B_{12} in diabetic retinopathy *Am J Ophth* 38 53–59 July 1954

146 Ralli E P Barbosa F X Dumm M E Beck E M and Laken B Prolonged administration of vitamin B_{12} folic acid and calcium pantothenate in patients with diabetes mellitus *J Clin Endocrinol & Metab* 15 898 July 1955 (in Soc Proc)

147 Sauer H and Dussler A On the disease picture of diabetic polyneuritis and its treatment with vitamin B_{12}, *Deutsche med Wchnschr* 79 1046–1048 June 25 1954 (abstr *Diabetes* 4 71 Jan–Feb 1955)

148 Lueck, A G Neurologic disturbances complicating diabetes *J Iowa M Soc* 46 296–300 June 1956

149 Coste P D and Klinger R Use of massive doses (500 to 1 000 mcg) of vitamin B_{12} for various conditions present in diabetic patients *Policlinico* (sez. prat) 61 143–149, Feb 1 1954 (abstr *J A M A* 155 781, June 19 1954)

150 Hirson, C Diabetic nocturnal diarrhea, *Lancet* 2 1227 Dec 29 1951 (in Letters to the Editor)

151 Alexander, E, Jr and Davis C H Jr Trigeminal neuralgias massive vitamin B_{12} therapy *North Carolina M J* 14 206–207 May 1953 (abstr *Clin Med* 61 400 May 1954)

152 Albrecht K and Krump J Diagnosis differential diagnosis and therapeutic possibilities in trigeminus neuralgia (conservative treatment with hydantoin drugs and vitamin B_{12}) [in German] *Munchen med Wchnschr* 96 1037–1039 Sept 3 1954

153 Surtees S J and Hughes R R Treatment of trigeminal neuralgia with vitamin B_{12} *Lancet* 1 439–441 Feb 27 1954

154 Fields W S and Hoff H E. Relief of pain in trigeminal neuralgia by crystalline vitamin B_{12} *Neurology* 2 131–139 March–April 1952

155 Jolles K E Herpes zoster treated with vitamin B_{12} *Brit M J* 1 959 April 22 1950 (in Correspondence)

156 Jolles K E Herpes zoster treated with vitamin B_{12} *Brit M J* 1 166–167, Jan 15, 1955 (in Correspondence)

157 Leitch G B Vitamin B_{12} in massive dosages for herpetic lesions a preliminary report *Northwest Med* 52 291–292, April 1953

158 Howard C Liver extract for herpetic pain *Lancet* 2 242 Aug 2 1952

159 Levin M B Vitamin B_{12} in neuro-metabolism (preliminary clinical report) *Am J Digest Dis* 22 96–97 April 1955

160 Lereboullet J and Pluvinage R Use of vitamin B_{12} in neurology *J A M A* 148 667, Feb 23 1952 (in Foreign Letters Paris)

161 Booth C B Lawyer T Jr and von Storch T J C Vitamin B_{12} in the treatment of multiple sclerosis *J A M A* 147 894 Oct 27 1951 (in Correspondence)

162 Schmidt W and Winter H Preliminary results with a high dosage vitamin B_{12} preparation (docigram 1000) [in German] *Ther d Gegenw* 93 265–267 July 1954 (abstr *Schweiz med Wchnschr* 85 137 Feb 5 1955)

163 Lereboullet, J and Pluvinage R Use of massive doses of vitamin B_{12} in neurology (1 000 micrograms per injection) *Semaine d hôp Paris* 29 1849–1854 June 6 1953 (abstr *J A M A* 153 601 Oct 10 1953)

164 Benbanasté J V Vitamin B_{12} in clinical neurology [in Spanish] *Prensa méd argent* 41 1666–1668 June 11 1954

165 Bacher K R Vitamin B_{12} for treatment of alcoholic delirium *Deutsche med Wchnschr* 79 1901–1903 Dec 17 1954 (abstr *J A M A* 157 969 March 12, 1955)

166 Menof P New treatment of polyneuritis *South African M J* 24 715–717 Aug 26 1950 (abstr *J A M A* 144 1521, Dec. 23 1950)

167 Menof P Role of vitamin B_{12} in polyneuritis fatty degeneration and pernicious anaemia *South African M J* 25 394–399 June 9 1951 (abstr *J A M A* 147 791 Oct 20 1951)

168 Gorman, W F Alcoholic neuritis *Ann Int Med* 37 566–573 Sept 1952.

169 Murphy G Alcoholic polyneuritis treatment with vitamin B_{12} [in Spanish] *Prensa med argent* 41 804–806 March 19 1954

170 Levy I The use of vitamin B_{12} in the symptomatic treatment of peripheral neuropathy preliminary report *Tr Am Neurol A* 75 267–270 June 1950

171 Natenshon A L A new form of drug therapy in the treatment of arthritis and rheumatoid conditions *Wisconsin M J* 53.223–225 April 1954

172 Norcross B M Lockie L. M and Talbott, J H Osteoarthritis *Gen Practitioner* 11·93–101 March 1955

173 Levy M D Management of chronic arthritis *Texas State J Med* 52 285–287 May 1956

174 Neuroblastoma *J.A M A* 159 393 Sept 24 1955 (in Foreign Letters England)

175 Daur W Vitamin B_{12} in the treatment of poliomyelitis [in German] *Munchen med Wchnschr* 96 729–730 June 18 1954

176 Daur W Vitamin B_{12} in the treatment of poliomyelitis [in German] *München med Wchnschr* 98 1366 Oct 5 1956

177 Shils M E. and Stewart W B Development of portal fatty liver in rats on corn diets response to lipotropic agents *Proc Soc Exper Biol & Med* 85 298–303 Feb 1954

178 Mallov S Effect of chronic ethanol intoxication on liver lipid content of rats *Proc Soc Exper Biol & Med* 88 246–249 Feb 1955

179 Bennett, M A Hellerman J and Donnelly A J Liver lesions due to prolonged feeding of a labile methyl free diet and the protective influence of vitamin B_{12} *Proc Am A Cancer Research* 1 4–5 April 1954

180 Schweigert, B S Scheid H E and Downing M Liver changes in vitamin B_{12} and riboflavin-deficient rats before and after partial hepatectomy *Am J Physiol* 178 338–340 Aug 1954

181 Ralli E. P Leslie S H Stueck G H Jr Shorr H E Robson J S Clarke D H and Laken B Course of cirrhosis of liver in patients treated with large doses of liver extract intravenously *Medicine* 28 301–332, June 11 1949

182 Sunder Rao A R Krishnaswamy Rao S and Anderson R K Vitamin B_{12} in infantile cirrhosis *Current Science* 20 41–42, Feb 1951

183 Doraiswamy T R Naidu V R Lakshminarayan Rao M V and Subrahmanayan V Vitamin B_{12} in infantile cirrhosis *Current Science* 20 183 July 1951

184 Campbell R E and Pruitt F W Vitamin B_{12} in the treatment of viral hepatitis a preliminary report *Am J M Sc* 224 252–262 Sept 1952

185 Campbell R E and Pruitt F W The effect of vitamin B_{12} and folic acid in the treatment of viral hepatitis *Am J M Sc* 229 8–15 Jan 1955

186 Colwell A R Jr Occurrence of accumulation of fat in the liver and its relation to excess weight gain in patients convalescing from viral hepatitis *Ann Int Med* 41 963–979 Nov 1954

187 Stevenson T D and Beard M D Observations on the metabolism of vitamin B_{12} in patients with liver disease *Clin Research Proc* 4 144 April 1956

188 Jones P N and Mills E H Serum vitamin B_{12} concentrations in liver disease *J Lab & Clin Med* 46 927 Dec 1955 (in Soc Proc)

189 Jones P N and Mills E H The effect of liver disease on serum vitamin B_{12} concentrations *J Clin Investigation* 35 716–717 June 1956 (in Soc Proc)

190 Rachmilewitz M Aronovitch J and Grossowicz N Serum concentrations of vitamin B_{12} in acute and chronic liver disease *J Lab & Clin Med* 48 339–344 Sept 1956

191 Jones, P N Mills, E H and Capps R B The effect of liver disease on serum vitamin B_{12} concentrations *J Lab & Clin Med* 49 910–922 June 1957

192 Glass G B J Ebin L M Boyd L J and Laughton R Hepatic uptake of radioactive vitamin B_{12} in liver disease *Federation Proc* 15 74–75 March 1956

193 Davis, R L, Duvall R C and Chow, B F Serum vitamin B_{12} level and binding substance of tuberculous patients with and without liver disease *J Lab & Clin Med* 49 422–428 March 1957

194 Baker, H Pasher I, Dolger H P and Sobotka H Vitamin B_{12} excretion as index of hepatic disorder *Clin Chem* 2 328–330 Oct 1956

195 Goldblatt, S Treatment of lupus erythematosus with vitamin B_{12} *J Invest Dermat* 17 303–304 Dec 1951

196 Block M T Vitamin E in the treatment of diseases of the skin, *Clin Med* 60 31–34 Jan 1953

197 Marcus M D Conrad A H Jr and Weiss R S Treatment of chronic discoid lupus erythematosus with large doses of vitamin B_{12} *J Invest Dermat* 21 75–77, Aug 1953 (in Preliminary and Short Reports)

198 Goldblatt S Cyanocobalamin (vitamin B_{12}) therapy of lupus erythematosus further observations *Acta dermat venereol* 33 216–235 1953

199 Andrews G C Post C F and Domonkos, A N Seborrheic dermatitis supplemental treatment with vitamin B_{12}, *New York State J Med* 50 1921–1925 Aug 15 1950

200 Simon S W Vitamin B_{12} therapy in allergy and chronic dermatoses *J Allergy* 22 183–185 March 1951

201 Krantz, J C, Jr and Carr, C J The Pharmacologic Principles of Medical Practice 3rd ed Baltimore, The Williams and Wilkins Company 1954, p 1038

202 Neber H The treatment of various skin diseases particularly those of allergic etiology with vitamin B_{12} [in German] *Munchen med Wchnschr* 97 1102–1103 Aug 26 1955

203 Robinson R C V Treatment of xanthelasma with vitamin B_{12} *J Invest Dermat* 24 111–113 Feb 1955

204 Ruedemann, R, Jr Treatment of psoriasis with large doses of vitamin B_{12} 1 100 micrograms per cubic centimeter, *A M A Arch & Syph* 69 738–739 June 1954 (in Clinical Notes New Instruments and Techniques)

205 Rimbaud P Ravoire J and Rioux J Treatment of psoriasis with vitamin B_{12} in a dose of 1000 mcg [in French] *Presse méd* 64 194, Feb 1 1956 (in Soc Proc)

206 Meites J Feng Y S L and Wilwerth A M The effects of endocrine imbalances on vitamin requirements *Am J Clin Nutrition* 5 381–392 July–Aug 1957

207 Strauss M B and Brokaw, R Adrenocortical function in pernicious anemia *New England J Med* 245 798–802 Nov 22, 1951

208 Sandberg A A, Eik Nes K, Nelson D H Palmer J G Cartwright G E and Wintrobe M M Adrenocortical function and metabolism of 17 hydroxycorticosteroids in pernicious anemia *New England J Med* 251 169–174 July 29 1954

209 Nutrition Note The anti thyrotoxic effects of vitamin B_{12} *Am J Digest Dis* 16 418 Nov 1949

210 Editorial Review Vitamin deficiencies in patients with diabetic retinopathy *Nutrition Rev* 13 74–76 March 1955 (abstr J Am Dietet A 31 514 516 May 1955)

211 Becker B Diabetic retinopathy *Ann Int Med* 37.273–289, Aug 1952

212 Friedenwald J S Diabetic retinopathy *Am J Ophth* 33 1187–1199 Aug 1950

213 Becker B Lang C A and Chow, B F Vitamin B_{12} excretion and diabetic retinopathy *Am J Clin Nutrition* 1 417–423 Sept–Oct 1953

214 Chow B F and Stone H H The relationship of vitamin B_{12} to carbohydrate metabolism and diabetes mellitus *Am J Clin Nutrition* 5 431–439 July–Aug 1957

215 Sebrell, W H Jr Adequate therapeutic diets *J Am Dietet A* 30 1256–1259 Dec 1954 (in Current Comment)

216 Wetzel N C Fargo W C Smith I H and Helikson, J Growth failure in school children as associated with vitamin B_{12} deficiency—response to oral therapy *Science* 110 651–653 Dec 16 1949

217 Wetzel N C Hopwood H H Kuechle M E and Grueninger R M Growth failure in school children Further studies of vitamin B_{12} dietary supplements *Am J Clin Nutrition* 1 17–31 Sept–Oct 1952

218 Jolliffe N Recent advances in nutrition of public health significance *Metabolism* 4 191–203 May 1955

219 Scrimshaw N S and Guzmán M A The effect of dietary supplementation and the administration of vitamin B_{12} and Aureomycin on the growth of school children, Nutrition Symposium Series No 7 National Vitamin Foundation Inc. New York 1953 pp 101–117

220 DeMezei, M B The effect of vitamin B_{12} in malnourished infants *Arch argent pediat* 37 31 1952 (abstr *A M A Am J Dis Child* 86 674 Nov 1953)

221 Larcomb J W., Perry C S and Peterman, R A Dietary supplementation of vitamin B_{12} in prepuberty school age children I Growth studies *J Pediat* 45 70–74 July 1954

222 Grump J and Tully R The use of partial vitamin supplements in the treatment of growth failure in children, *J Pediat* 46 671–681 June 1955

223 Bidault Activity of vitamin B_{12} given orally as a growth factor in the child [in French] *Concours med* 73 3673–3674 Nov 3 1951

224 Scrimshaw N S Tandon, O B and Pérez, C. Effect of vitamin B_{12} Aureomycin and penicillin on growth of Guatemalan school children *Federation Proc* 15 572, March 1956

225 Patrick S J Some observations on the metabolism of vitamin B_{12} by Jamaican children *J Nutrition* 55 129–135 Jan 1955

226 Mackay I F S Patrick S J Stafford D and Cleveland F S Jr The influence of vitamin B_{12} and Aureomycin upon the growth of protein-deficient children, *J Nutrition* 59 155–170 May 1956

227 Campbell J A and McLaughlan J M Vitamin B_{12} and the growth of children a review *Canad M A J* 72 259–263 Feb 15 1955

228 Chow B F Sequelae to the administration of vitamin B_{12} to humans *J Nutrition* 43 323–343 Feb 1951

229 Chow B F The role of vitamin B_{12} in metabolism *South M J* 45 604–612 July 1952

230 Masi A and Mori S Therapeutic activity of liver extracts and vitamin B_{12} in children *Minerva pediat* 5 290–291 April 30 1953 (abstr *J A M A* 153 1129 Nov 21 1953)

231 Kofman I General stimulating effect of vitamin B_{12} in pediatrics [in Spanish] *Semana méd* 2 261–266 Aug 20 1953

232 Kofman I Massive doses of vitamin B_{12} for poor appetite in childhood [in Spanish] *Orientacion med* 2 1204–1210 Aug 27 1954

233 Grüninger U., Bamberger G and Piening A The value of cytobion as an appetite and stamina raising agent [in German] *München med Wchnschr* 98 55–57 Jan 13 1956

234 Hellegers A Okuda K Nesbit R E L Jr Smith B W and Chow B F Vitamin B_{12} absorption in pregnancy and in the newborn *Am J Clin Nutrition* 5 327–331 May–June 1957

235 Boger W P Wright L D Back G D and Bayne G M Vitamin B_{12} correlation of serum concentration and pregnancy *Proc Soc Exper Biol & Med* 92 140–143 1956

236 Baker H Erdberg R and Sobotka H Vitamin B_{12} in serum and urine in pregnancy *Clin Chem* 2 291 Aug 1956 (in *Soc Proc*)

237 Okuda K Helliger A E and Chow B F Vitamin B_{12} serum level and pregnancy *Am J Clin Nutrition* 4 440–443 July–Aug 1956

238 Boger W P Bayne G M Wright L D and Beck G D Differential serum vitamin B_{12} concentrations in mothers and infants *New England J Med* 256 1085–1087 June 6 1957

239 Jones C C Brown S O Richardson L R and Sinclair J G Tissue abnormalities in newborn rats from vitamin B_{12} deficient mothers *Proc Soc Exper Biol & Med* 90 135–140 1955

240 Grainger R B O Dell B L and Hogan A G Congenital malformations as related to deficiencies of riboflavin and vitamin B_{12} source of protein, calcium to phosphorus ratio and skeletal phosphorus metabolism *J Nutrition* 54 33–48 Sept 1954

241 Machella T E Masked and obvious vitamin deficiency states in older age *Pennsylvania M J* 58 587–590 June 1955
242 Kaufman W The use of vitamin therapy to reverse certain concomitants of aging *J Am Geriatric Soc* 3 927–936, Nov 1955
243 Fabricant N D Geriatric otolaryngology *Am J M Sc* 232 452–457 Oct 1956
244 Chow B F Vitamin B_{12} and aging *Federation Proc* 13 453–454 March 1954
245 Chow B F The absorption of vitamins and enzymic activities in aging *Internat Rec Med* 167 429–430 July 1954 (in Soc Proc)
246 Tauber, S A Goodhart, R S, Hsu J M, Blumberg N Kassab J and Chow, B F Vitamin B_{12} deficiency in the aged *Geriatrics* 12 368–374 June 1957
247 Boger, W P, Wright L D Strickland, S C Gylfe J S and Ciminera J L Vitamin B_{12} correlation of serum concentrations and age *Proc Soc Exper Biol & Med* 89 375–378 July 1955
248 Chow B F Wood R Horonick, A and Okuda K Agewise variation of vitamin B_{12} serum levels *J Gerontol* 11 142–146 April 1956
249 Gaffney G W Horonick A, Okuda K Meier P Chow B F and Shock N W Vitamin B_{12} serum concentrations in 528 apparently healthy human subjects of ages 12 to 94 *J Gerontol* 12 32–38, Jan 1957
250 Zintel H A Nutritional rehabilitation of the elderly patient undergoing surgery *Am J Clin Nutrition* 3 501–510 Nov–Dec 1955
251 Schiller F Herpes zoster review with preliminary report on new method for treatment of postherpetic neuralgia *J Am Geriatrics Soc* 2 726–735 Nov 1954
252 Marks M M Nonspecific ulcerative colitis antibiotic residues in treatment *Missouri Med* 51 189–190 March 1954
253 Vitamin B_{12} *J A M A* 153 257 Sept 19 1953 (in Queries and Minor Notes)
254 Pollack, H and Halpern, S L Therapeutic Nutrition, Washington D C National Research Council Publication 234 1952 pp 36–37

Part 3

Selected Annotated Bibliography

INTRODUCTION

This annotated bibliography comprises abstracts of selected material appearing in the medical and related scientific literature since July 1953 In choosing many of the abstracts an attempt has been made to present recent reports on the established clinical uses of vitamin B_{12} and also to draw attention to the various diseases and disorders in which vitamin B_{12} has been reported to have therapeutic or nutritional application This bibliography is designed to supplement the comprehensive collection of abstracts and reference data presented in the Merck Service Bulletin on Vitamin B_{12}, Part 2, Annotated Bibliography, published in 1954

MACROCYTIC ANEMIAS GENERAL

1 Nelson N A and Bishop, R C. Nonaddisonian megaloblastic anemia Clin Research Proc. 4 234 Nov 1956 (In Soc. Proc.)

Pernicious anemia either in relapse or remission, may now be diagnosed by demonstrating the absence of intrinsic factor activity by means of radioactive vitamin B_{12} studies Such studies may also be of help in the diagnosis of pernicious anemia in patients with neurologic disease resembling subacute combined degeneration without anemia or in those who have received inadequate therapy

Recently radioactive vitamin B_{12} was used to study 2 patients with macrocytic megaloblastic anemia and histamine fast achlorhydria One had a history of dietary insufficiency and purpuric skin lesions suggesting scurvy After the Schilling test effectively excluded the diagnosis of pernicious anemia he improved without therapy other than diet The other patient was an epileptic on Dilantin An adequate hematologic response occurred in this patient during a trial with investigational material The Schilling test performed on 2 occasions however demonstrated normal urinary excretion of radioactive vitamin B_{12} again eliminating the diagnosis of addisonian pernicious anemia and the need for continued vitamin B_{12} therapy

In a third patient with nutritional megaloblastic anemia free hydrochloric acid was present in the gastric juice A radioactive vitamin B_{12} excretion test confirmed the presence of intrinsic factor activity

Wayne County Gen al Hospital
Eloise Mich

2 Mollin D L and Ross G I M Serum vitamin B_{12} concentrations of patients with megaloblastic anemia after treatment with vitamin B_{12}, folic acid or folinic acid Brit M J 2 640-645, Sept 19 1953

Single intramuscular injections of 20 to 1 000 mcg of vitamin B_{12} were given to 33 patients with pernicious anemia in relapse Concentrations of vitamin B_{12} in serum (as shown by Euglena gracilis assay) rose from low pretreatment levels and remained within normal range for lengths of time somewhat dependent on the size of the dose e g 6 to more than 18 days after 20 mcg 11 to at least 35 days after 80 mcg 34 to at least 58 days after 1 000 mcg Although concentrations were within the normal range for these periods the mean of the concentrations of all patients in each group fell below the average found in normal subjects within a few days of injection

Serum vitamin B_{12} levels were maintained within the normal range in most patients who received 40 mcg of the vitamin every 10 days or 160 mcg every 21 days The authors state that these doses appear to be the minimum requirement for patients in relapse but they recommend that large doses (up to 5 mg) be given in the first week or two

When serum vitamin B_{12} concentrations were normal hemopoiesis was normoblastic when concentrations were below normal megaloblasts reappeared in the marrow In 12 patients with megaloblastic anemia of sprue or pregnancy serum concentrations of vitamin B_{12} were normal Treatment of these patients with vitamin B_{12} was ineffective and treatment with folic or folinic acid which was effective did not affect serum levels of vitamin B_{12}

Postgraduate Medical School of London
London England

3 Nieweg H O Faber, J G de Vries J A and Kroese, W F S The relationship of vitamin B_{12} and folic acid in megaloblastic anemias, J Lab & Clin Med 44 118 132, July 1954

Patients with megaloblastic anemia who have neurologic disorders respond to vitamin B_{12} therapy Such patients have low serum levels of this vitamin and normal or low folic acid activity of whole blood Patients with megaloblastic anemia who respond only to folic acid have normal or high serum levels of vitamin B_{12} and low whole blood folic acid activity

In 7 untreated cases of pernicious anemia intramuscular injections of vitamin B_{12} caused a decrease in the folic acid activity of the blood The value rose again after 72 hours A review of theories and data concerning folic acid metabolism in pernicious anemia led to the conclusions that vitamin B_{12} administration causes an increased utilization of folic acid in the bone marrow, has some influence on its storage in the liver and possibly causes an increased intake of folic acid

The function of folic acid and vitamin B_{12} in nucleic acid synthesis is discussed The authors suggest that vitamin B_{12} is necessary for the production of both ribonucleic acid (RNA) and desoxyribonucleic acid (DNA) while folic acid participates only in the synthesis of the latter Since RNA takes part in protein synthesis and DNA is a constituent of the chromosomes in the nuclei of cells deficiency of vitamin B_{12} produces both lesions of the nervous system (RNA) and hematologic disorders (DNA) while folic acid deficiency causes only the latter The authors emphasize that this is theoretical speculation only

State University of Groningen
Groningen Netherlands

4 Lipetz S Prostatic carcinoma and macrocytic anemia Lancet 2 498 Sept 4, 1954 (Letter to Editor)

Vitamin B_{12} was 81 year old man for macro de

veloped about two years after the of the prostatic carcinoma Both a and urinary symptoms disappeare matically soon after vitamin B_{12} th was begun

The case is presented as releva the hypothesis that there are possibl between cancer and other metabolic esses because (1) Pernicious an has its associated neuropathy and a association with gastric carcinom There is reason to suppose that the some unknown factors interfering haemoglobin synthesis and red-cel mation in the presence of certain plasms (3) Animal experiment ha gested that vitamin B_{12} might be eff in the treatment of some cases of r nant disease and it has recently be ported that several cases of neuroblas have shown remarkable and lengt gression after treatment with vitamin

Edinburgh Scotland

5 Mueller J F and Will J J I relationship of folic acid vitam and ascorbic acid in patients megaloblastic anemia Am J Nutrition 3 30-44, Jan Feb 195

The authors discuss the chemical relationships of vitamin B_{12} and foli in bringing about the maturation c pernicious anemia megaloblast It is posed that vitamin B_{12} and folic ac as catalysts in a reaction leading t formation of nucleic acid and that a bic acid affects folic acid metaboli does vitamin B_{12}, a deficiency of vit B_{12} will result in a conditioned defic of folic acid Clinical experiments formed to test this hypothesis are scribed

The authors conclude that folic

In pernicious anemia folic acid therapy temporarily corrects the defect in nucleoprotein metabolism by mass action Eventually a greater deficiency of vitamin B_{12} occurs, resulting in hematologic relapse and progressive neurologic disease

Pernicious anemia of pregnancy and vitamin B_{12} refractory megaloblastic anemia also occur because of defects in nucleoprotein metabolism These anemias are caused by deficiencies of folinic acid coenzyme Ascorbic acid metabolism also may be altered in patients with megaloblastic anemias since vitamin C is capable of producing reticulocytosis and improvement in some of these patients

University of Cincinnati
College of Medicine
Cincinnati Ohio

6 Vilter R. W Treatment of macrocytic anemias A M A Arch Int Med 95 482-492 March 1955 (in Treatment in Internal Medicine)

The author reviews the literature on macrocytic anemias and discusses their differential diagnosis For therapy in uncomplicated pernicious anemia he compares the parenteral use of vitamin B_{12} and refined liver extract and states that vitamin B_{12} is cheaper and causes so few reactions that it is the preferred agent There is no evidence that liver extract will induce any better response than vitamin B_{12} nor is there any evidence that folic acid ascorbic acid or iron is needed in the average case He does not favor the oral vitamin B_{12}-intrinsic factor preparation Specific recommendations for vitamin B_{12} dosage are given Iron is recommended when hypochromia is present folic acid is recommended when a dietary inadequacy is persistent and anemia reappears in spite of vitamin B_{12} therapy Vitamin B_{12} is recommended in nutritional macrocytic anemia and sprue although supplementary folic acid may be required in the latter disease

University of Cincinnati
College of Medicine
Cincinnati Ohio

7 Unglaub W G and Goldsmith G A Oral vitamin B_{12} in the treatment of macrocytic anemias South M J 48 261 269 March 1955

Large oral doses of vitamin B_{12} were given to 11 patients with pernicious anemia and to 3 patients with nutritional macrocytic anemia Dosages varied from 3 000 mcg every four weeks to 1 000 mcg weekly One patient received 3 000 mcg weekly Maintenance therapy continued for from 4 to 28 months Of the patients with pernicious anemia 5 had received no previous therapy the other 6 had previously received various types of therapy

Satisfactory hematologic response was seen in all patients Serum vitamin B_{12} in 9 increased from pretreatment levels of 0 02 to 0 05 millimicrograms per cc to levels of 0 10 to 0 68 millimicrograms per cc In 3 pernicious anemia patients with subacute combined sclerosis of the spinal cord marked improvement followed oral vitamin B_{12} therapy Of the patients with nutritional macrocytic anemia 2 relapsed after vitamin B_{12} therapy was withdrawn in spite of diets considered nutritionally adequate One had a decrease in serum vitamin B_{12} levels paralleling the fall in the blood count

Tulane University School of Medicine and
Charity Hospital of Louisiana
New Orleans La

8 Watkins C H The treatment of anemia Minnesota Med 38 327 330 May 1955

Effective doses of liver extract or vitamin B_{12} are important in the treatment of pernicious anemia Initial doses of 45 units liver extract or 60 mcg vitamin B_{12} are given intramuscularly daily for three days followed by 15 units liver extract or 30 mcg vitamin B_{12} once a week until the blood picture becomes normal Maintenance doses are continued for life Responses to liver extract and vitamin B_{12} are essentially the same Vitamin B_{12} has

3 Nieweg H O, Faber, J G de Vries, J A and Kroese W F S The relationship of vitamin B_{12} and folic acid in megaloblastic anemias J Lab & Clin Med 44 118-132 July 1954

Patients with megaloblastic anemia who have neurologic disorders respond to vitamin B_{12} therapy Such patients have low serum levels of this vitamin and normal or low folic acid activity of whole blood Patients with megaloblastic anemia who respond only to folic acid have normal or high serum levels of vitamin B_{12} and low whole blood folic acid activity

In 7 untreated cases of pernicious anemia intramuscular injections of vitamin B_{12} caused a decrease in the folic acid activity of the blood The value rose again after 72 hours A review of theories and data concerning folic acid metabolism in pernicious anemia led to the conclusions that vitamin B_{12} administration causes an increased utilization of folic acid in the bone marrow has some influence on its storage in the liver, and possibly causes an increased intake of folic acid

The function of folic acid and vitamin B_{12} in nucleic acid synthesis is discussed The authors suggest that vitamin B_{12} is necessary for the production of both ribonucleic acid (RNA) and desoxyribonucleic acid (DNA) while folic acid participates only in the synthesis of the latter Since RNA takes part in protein synthesis and DNA is a constituent of the chromosomes in the nuclei of cells deficiency of vitamin B_{12} produces both lesions of the nervous system (RNA) and hematologic disorders (DNA) while folic acid deficiency causes only the latter The authors emphasize that this is theoretical speculation only

State Uni ersity of Groningen Groningen Netherlands

4 Lipetz S Prostatic carcinoma and macrocytic anemia Lancet 2 498 Sept 4, 1954 (Letter to Editor)

Vitamin B_{12} was given to an 81 year old man for macrocytic anemia that developed about two years after the onset of the prostatic carcinoma Both anemia and urinary symptoms disappeared dramatically soon after vitamin B_{12} therapy was begun

The case is presented as relevant to the hypothesis that there are possible links between cancer and other metabolic processes because (1) Pernicious anaemia has its associated neuropathy and a direct association with gastric carcinoma (2) There is reason to suppose that there are some unknown factors interfering with haemoglobin synthesis and red-cell formation in the presence of certain neoplasms (3) Animal experiment has suggested that vitamin B_{12} might be effective in the treatment of some cases of malignant disease and it has recently been reported that several cases of neuroblastoma have shown remarkable and lengthy regression after treatment with vitamin B_{12}

Edinburgh Scotland

5 Mueller, J F and Will, J J Interrelationship of folic acid vitamin B_{12} and ascorbic acid in patients with megaloblastic anemia Am J Clin Nutrition 3 30-44 Jan Feb 1955

The authors discuss the chemical interrelationships of vitamin B_{12} and folic acid in bringing about the maturation of the pernicious anemia megaloblast It is proposed that vitamin B_{12} and folic acid act as catalysts in a reaction leading to the formation of nucleic acid and that ascorbic acid affects folic acid metabolism as does vitamin B_{12} a deficiency of vitamin B_{12} will result in a conditioned deficiency of folic acid Clinical experiments performed to test this hypothesis are described

The authors conclude that folic acid and vitamin B_{12} deficiency states are associated with defects in nucleoprotein metabolism characterized biochemically by increased ratios of ribonucleic acid to desoxyribonucleic acid and uracil to thymine These biochemical changes are correlated with the degree of megaloblastosis

In pernicious anemia folic acid therapy temporarily corrects the defect in nucleoprotein metabolism by mass action Even tually a greater deficiency of vitamin B_{12} occurs resulting in hematologic relapse and progressive neurologic disease

Pernicious anemia of pregnancy and vitamin B_{12} refractory megaloblastic anemia also occur because of defects in nucleoprotein metabolism These anemias are caused by deficiencies of folinic acid coenzyme Ascorbic acid metabolism also may be altered in patients with megaloblastic anemias since vitamin C is capable of producing reticulocytosis and improvement in some of these patients

University of Cinci nati
Coll ge of Medicine
Cincinnati Ohi

6 Vilter R W Treatment of macrocytic anemias A.M A Arch Int Med 95 482-492 March 1955 (in Treatment in Internal Medicine)

The author reviews the literature on macrocytic anemias and discusses their differential diagnosis For therapy in un complicated pernicious anemia he compares the parenteral use of vitamin B_{12} and refined liver extract and states that vitamin B_{12} is cheaper and causes so few reactions that it is the preferred agent There is no evidence that liver extract will induce any better response than vitamin B_{12} nor is there any evidence that folic acid ascorbic acid or iron is needed in the average case He does not favor the oral vitamin B_{12} intrinsic factor preparation. Specific recommendations for vitamin B_{12} dosage are given Iron is recommended when hypochromia is present folic acid is recommended when a dietary inadequacy is persistent and anemia re appears in spite of vitamin B_{12} therapy Vitamin B_{12} is recommended in nutritional macrocytic anemia and sprue although supplementary folic acid may be required in the latter disease

7 Unglaub W G and Goldsmith G A Oral vitamin B_{12} in the treatment of macrocytic anemias South M J 48 261 269, March 1955

Large oral doses of vitamin B_{12} were given to 11 patients with pernicious anemia and to 3 patients with nutritional macrocytic anemia Dosages varied from 3 000 mcg every four weeks to 1 000 mcg weekly One patient received 3 000 mcg weekly Maintenance therapy con tinued for from 4 to 28 months Of the pati nts with pernicious anemia 5 had received no previous therapy the other 6 had previously received various types of therapy

Satisfactory hematologic response was seen in all patients Serum vitamin B_{12} in 9 increased from pretreatment levels of 0 02 to 0 05 millimicrograms per cc t levels of 0 10 to 0 68 millimicrograms p cc In 3 pernicious anemia patients wi subacute combined sclerosis of the spir cord marked improvement followed o vitamin B_{12} therapy Of the patients w nutritional macrocytic anemia 2 relap after vitamin B_{12} therapy was withdr in spite of diets considered nutritio adequate One had a decrease in s vitamin B_{12} levels paralleling the f the blood count

T lane University School of Medicine and
Cha ity Hospital of Louisi na
New Orleans La

8 Watkins C H The treatr anemia Minnesota Med 38 May 1955

Effective doses of liver extra min B_{12} are important in the tr pernicious anemia Initial d units liver extract or 60 mcg are given intramuscularly da days followed by 15 units liv 30 mcg vitamin B_{12} once a w blood picture becomes nor nance doses are continued sponses to liver extract an are essentially the same V

however an advantage over liver extract in that it is a purified crystalline substance of constant potency which may be used in patients sensitive to liver extract it produces a minimal local reaction Folic acid citrovorum factor, liver extract combined with iron and hydrochloric acid although not contraindicated are not preferred methods of therapy

Other types of megaloblastic anemia are often difficult to treat If no response occurs after two or three weeks of vitamin B_{12} therapy folic acid administration should be tried then citrovorum factor Occasionally a rare macrocytic anemia with a relatively aplastic bone marrow responds to cortisone then to folic acid or vitamin B_{12} In refractory anemias such treatment might be tried

Mayo Foundation and Mayo Clinic
Rochester Minn

9 Girdwood, R H The megaloblastic anemias Their investigation and classification, Quart J Med 25 87-119 1956 (abstr Blood 11 1053, Nov 1956)

This account seeks to classify the megaloblastic anemias largely from estimation of folic acid absorption and of the serum vitamin B_{12} level Pernicious anemia may be diagnosed by the serum vitamin B_{12} estimation before megaloblastic anemia develops The folic acid absorption test showed malabsorption to be present in 28 cases of idiopathic steatorrhea investigated whether or not megaloblastic anemia was present This malabsorption was not present in other forms of megaloblastic anemia steatorrhea and megaloblastic anemia after gastrectomy or gastroenterostomy were associated with vitamin B_{12} deficiency but not with malabsorption of folic acid Megaloblastic anemia of pregnancy was not associated with vitamin B_{12} deficiency or with folic acid malabsorption but there are several types of megaloblastic anemia that may occur in pregnancy When a patient with vitamin B_{12} deficiency is treated with folic acid for a long period megaloblastosis may recur

University of Edinburgh
Edinburgh Scotland

PERNICIOUS ANEMIA

Pernicious Anemia Diagnostic Studies

10 Graham R M and Rheault M H Characteristic cellular changes in epithelial cells in pernicious anemia J Lab & Clin Med. 43 235 245, Feb 1954

Specific cellular changes occurring in the epithelial cells of 41 pernicious anemia patients were studied Twenty patients were in relapse and 21 were in therapeutic remission All patients had severe macrocytic anemia before treatment and all showed a therapeutic response to vitamin B_{12} or liver therapy All had histamine fast achlorhydria Definite changes in the squamous epithelial cells the columnar epithelial cells and the histiocytes found in the gastric secretions of patients with untreated pernicious anemia and patients in relapse were observed During treatment these changes markedly decreased and were almost completely absent in patients in clinical remission following vitamin B_{12} or liver therapy

Though this study was mainly concerned with the cellular picture of the gastric secretion specific abnormal cell forms were also found in the sputum in smears from the oral cavity and in the vaginal smear The suggestion is made that the extrinsic factor (vitamin B_{12}) may be essential for the normal maturation of epithelial cells just as it is for the normal maturation of the red blood cells

Vincent Memorial Hospital and
Massachusetts General Hospital
Boston Mass

11 Weaver J A and Neill D W Amino-aciduria in pernicious anemia and subacute combined degeneration of the cord Lancet 2 1212 1213, June 12 1954

Five patients with pernicious anemia showed abnormal excretion of taurine lysine cystine and leucine which reverted toward normal after treatment with vitamin B_{12} Five patients with other types of anemia showed no abnormal amino-acid pattern in the urine The damage caused or the area affected by the lack of vitamin B_{12} in patients with pernicious anemia has not been determined

One case of subacute combined degeneration of the cord is discussed in detail Before treatment there was excessive excretion of taurine glycine alanine β amino-iso-butyric acid and glutamic acid Vitamin B_{12} 100 mcg daily was given intramuscularly for 14 days followed by 100 mcg twice weekly After this treatment the excessive taurine excretion ceased

The possible causes of amino-aciduria in pernicious anemia are discussed and it is suggested that because vitamin B_{12} is involved in the transmethylation of certain amino acids a failure of this action in pernicious anemia may be the basis of development of megaloblastic bone marrow It is stated that further study of the amino-aciduria of untreated pernicious anemia may help determine the basic action of vitamin B_{12}

The authors suggest that the association of achylia gastrica and increased taurine excretion in the urine may permit early diagnosis of subacute combined degeneration of the cord

Royal Victoria Hospital and Queen's University Belfast North I eland

12 Conley C L Pitfalls in the diagnosis of pernicious anemia Gen Practitioner 11 59-61, Jan 1955

When a patient with symptoms of pernicious anemia responds dramatically to the parenteral administration of vitamin B_{12} convincing support for this diagnosis is provided When folic acid alone is administered to such a patient the hematologic disorders may clear completely while neurologic degeneration continues Thus in doubtful cases a thorough diagnosis must be made before any folic acid is administered The author states that deficiency of folic acid is extremely rare in the United States and that its indiscriminate use is not justified in view of the difficulties it causes in the diagnosis of other diseases

Preparations for oral use that combine vitamin B_{12} and the intrinsic factor are not as effective as vitamin B_{12} injected parenterally and should be considered experimental An injection of 50 mcg of vitamin B_{12} given once each six weeks constitutes good maintenance therapy When a patient on this maintenance dose has a relapse the presence of another disease (for example carcinoma of the stomach with hemorrhage into the gastrointestinal tract and subsequent anemia) must be considered

Johns Hopkins U iversity and Hospital Baltimore Md

13 Reisner D J and Ellsworth R M Coexistent idiopathic hypoparathyroidism and pernicious anemia in a young girl case report Ann Int Med 43 1116-1124 Nov 1955

In a child with hypoparathyroidism and pernicious anemia correct diagnosis was established by the urinary phosphorus response to intravenous parathyroid hormone and by a diagnostic trial of daily oral doses of 5 mcg of vitamin B_{12} The latter produced no reticulocyte response in eight days but reticulocyte response did occur when intrinsic factor was given with oral vitamin B_{12} Anemia improved on a regimen of 50 mcg of vitamin B_{12} given intramuscularly twice a week The patient is being adequately maintained on a monthly intramuscular dose of 30 mcg of vitamin B_{12} together with dihydrotachysterol calcium gluconate and viosterol

St Luke's Hospital New Yo k N Y

14 Rabiner, S F, Lichtman, H C, Messite, J Watson, R J, Ginsberg V, Ellenbogen, L and Williams, W L The urinary excretion test in the diagnosis of addisonian pernicious anemia, Ann Int Med 44 437-445, March 1956

The urinary excretion test with Co^{60} vitamin B_{12} was evaluated as a diagnostic procedure in pernicious anemia patients in hematologic remission This test was *also done in 24 control subjects in 36 pa*tients with pernicious anemia and in 33 pernicious anemia patients who were given intrinsic factor The procedure was to collect daily urinary output, the first day s specimen serving as control On the second day 2 mcg of Co^{60} vitamin B_{12} was given orally before breakfast and simultaneously 1 000 mcg of nonradioactive vitamin B_{12} was given intramuscularly (flushing dose) On the third day 1 000 mcg of vitamin B_{12} was injected On the fourth day intrinsic factor was given orally to 33 of the patients in addition to another intramuscular dose of 1,000 mcg of vitamin B_{12} The results are shown in a table

It is diagnostically significant that there is no overlap between the control group and the pernicious anemia patients not receiving intrinsic factor Thus pernicious anemia may be definitely diagnosed or ruled out In 26 of the 33 patients given intrinsic factor the percentage of urinary excreted radioactive vitamin B_{12} increased to within the normal range

State University of New York College of Medicine at New Yo k City and
Kings County Hospital
B ooklyn N Y

Lederle Laboratories
Pearl River N Y

15 Adams J F Glossitis and the pre anaemic stage of pernicious anaemia Diagnosis by simple methods Lancet 1 1120-1121, June 1 1957

Diagnosis of the pre anemic stage of pernicious anemia may be established by gastric aspiration bone marrow biopsy and response to vitamin B_{12} therapy Suspicion is first aroused by symptoms such as glossitis or neuropathy

The author presents histories of 3 patients with glossitis not accompanied by anemia All 3 had histamine fast achlorhydria and a sternal marrow that showed hyperplastic megaloblastic erythropoiesis Microbiologic assay revealed serum vitamin B_{12} levels of 40 45 and 120 micromicrograms per cc (normal mean 360 micromicrograms) Parenteral vitamin B_{12} (dosage not stated) abolished the glossitis but did not alter the blood levels in the first patient

Only one other comparable case has been reported in the literature but the syndrome may not be rare for the cases reported here constitute half the cases of glossitis without anemia seen in a year in a general medical unit They demonstrate *clearly that* the diagnosis of pernicious anemia can be established in the pre anemic stage

Western Infirmary
Glasgow Scotland

16 Rubin C E Phelps, P, Taniguchi, L and Fein, B The pathogenesis of the precancerous gastric lesion in pernicious anemia—a new theory, Clin Research Proc 4 199 200, Sept 1956

The authors state that deficient intestinal absorption of Co^{60} vitamin B_{12} offers confirmation of pre-anemic pernicious anemia sometimes suggested by exfoliated gastric cells The cytologic study of a gastric polyp led to the discovery of a pre anemic state in an achlorhydric patient Co^{60} vitamin B_{12} studies established the diagnosis in spite of normal neurologic and hematologic findings

The authors conclude 'The hereditary nature of PA [pernicious anemia] is established Some believe that gastric atrophy causes the characteristically defective B_{12} absorption It is possible that the only inherited trait is a diminished ability to manufacture intrinsic factor Thus gastric mucosal disease may be the result of B_{12} deficiency rather than its

cause Similarly the increased incidence of gastric polyp and carcinoma in PA may well be related to protracted B_{12} deficiency

Seattle Wash

17 Kristensen H P O and Olesen A S Early diagnosis of pernicious anemia with report of case Ugesk f laeger 118 1228-1231, Oct 18 1956 (abstr J.A M A. 163 494 Feb 9 1957)

A case is described in which moderately grave achylic iron deficiency anemia was accompanied by incipient pernicious anemia Treatment with iron fully normalized the hemoglobin percentage but the vitamin B_{12} content of the plasma was reduced Subsequent bone marrow examinations revealed distinct megaloblastic changes which disappeared after parenteral treatment with vitamin B_{12} Recognition of megaloblastic changes in the bone marrow in early pernicious anemia may be difficult If such changes are suspected in patients with signs of iron deficiency the plasma vitamin B_{12} level should be determined and examination of the bone marrow repeated after adequate treatment with iron

18 Hanlon D G Dodge H W Jr Siekert R. G and Bull F E Tumors of the spinal cord Occurrence in patients with pernicious anemia and subacute combined sclerosis J A M A 162 707 709 Oct 20 1956

Vitamin B_{12} was given parenterally to 2 patients with pernicious anemia and dorsolateral spinal degeneration Response was excellent but despite adequate maintenance therapy neurologic symptoms recurred in 1 patient after 13 months and in the other after 6 years When the symptoms failed to respond to intensified vitamin B_{12} therapy some cause of the neurologic symptoms other than pernicious anemia was sought It was found that both patients had tumors (meningioma and neurofibroma) of the spinal cord as well as pernicious anemia Prior to removal of the tumors the diagnosis of pernicious anemia in both patients was confirmed by the oral absorption test with radioactive vitamin B_{12}

Mayo Cli ic and Mayo Foundation Rochester Minn

Pernicious Anemia Treatment

19 Cameron D G, Townsend S R and English A Pernicious anaemia II Maintenance treatment with crystalline vitamin B_{12} Canad M A J 70 398-400 April 1954

Twenty six patients with pernicious anemia who had been satisfactorily maintained by intramuscular injection of 30 U S P units of concentrated liver extract weekly and 2 others who had been treated with vitamin B_{12} were satisfactorily maintained on weekly injections of 30 mcg of vitamin B_{12} The period of observation covered three to four years in 24 cases and over a year in 2 The results were comparable to those in 22 similar patients whose treatment was continued on liver extract

McGill Uni ersity Clinic of The Montre l Gene al Hospital Montreal Canada

20 Schwartz S O, Friedman I A and Gant, H L Long term evaluation of vitamin B_{12} in treatment of pernicious anemia I Incidental report on use of combined oral therapy with vitamin B_{12} and folic acid J A M A 157 229 231 Jan 15 1955

This long term evaluation of 32 patients with pernicious anemia treated with parenteral vitamin B_{12} and 34 patients treated with liver extract covers a period of at least four years The dosage of vitamin B_{12} was 30 mcg every four weeks the dosage of liver extract was 30 units each month

In 4 patients neurologic relapse occurred after 4 6 13 and 28 months of vitamin B_{12} therapy Of these patients 3 improved when vitamin B_{12} dosage was

increased another, whose relapse was severe improved after two months of weekly liver injections None of the patients had hematologic relapses while receiving vitamin B_{12}

Among the 34 patients treated with liver extract 3 had slight recurrence of paresthesias after 29, 30 and 20 months but all these patients responded favorably to increased doses of liver extract

Oral treatment of pernicious anemia with 25 mcg of vitamin B_{12} and 1 67 mg of folic acid given daily to 36 patients, led to hematologic neurologic or symptomatic relapse in 14, after periods varying from 15 to 49 months of therapy

The authors conclude that combined oral administration of vitamin B_{12} and folic acid is inadequate in pernicious anemia but that parenteral vitamin B_{12} is an adequate substitute for liver Neurologic relapse can be prevented with more frequent doses of vitamin B_{12}

Hektoen Institute for Medical Research of Cook County Hospital Chicago Ill

21 Prognosis in treated anaemia, Brit M J 1 297 298 Feb 2 1957 (in Any Questions?)

Q —*What is the prognosis for patients with pernicious anaemia treated with adequate amount of vitamin B_{12}? Are they ever cured in the sense that treatment is no longer necessary?*

A —The introduction of vitamin B_{12} (cyanocobalamin) is too recent for reliable statistics on survival and morbidity among patients with pernicious anaemia treated with the pure vitamin Clinical observation however suggests that such patients have an expectation of life and a morbidity no worse than that of the general population in fact it has been suggested that they may be better off because they come under review every month or two when they receive their injections

In his presidential address to the Manchester Medical Society last October Dr J F Wilkinson reviewed a personal series of 2 211 cases of pernicious anaemia seen over the past 28 years Of course, only in the last few years would pure vitamin B_{12} have been available for the treatment of these patients but the mortality in this series was only 0 83 per cent per year Almost all died from conditions other than pernicious anaemia but, of those who died of cancer, in about half the primary [neoplasm] was in the stomach In Dr Wilkinson s experience the expectation of life for the patients in this regularly supervised group of patients was rather better than for people without pernicious anaemia Of the 1 550 patients still attending his clinic no fewer than 758 were over 70 years of age 283 over 80 years 39 over 90 and one over 100

Patients with pernicious anaemia are never cured in the strict sense of the word, though if adequately treated their lives are in every respect as if they had been The chief pathological change is the atrophy of the gastro intestinal mucosa which so far has proved irreversible Very occasionally a patient who discontinues treatment remains well for months or even years before relapsing but these are exceptional cases and afford no general grounds for the discontinuance of treatment Treatment must be for life '

22 Dosage of vitamin B_{12} J A M A 163 998, March 16, 1957 (in Queries and Minor Notes)

A physician inquires whether vitamin B_{12} in doses of 1 000 mcg is more effective than in doses of 100 mcg

Answer Intramuscularly given doses of 1 000 mcg (1 mg) of cyanocobalamin are far in excess of those required in the proper treatment of pernicious anemia ' The paper written for The Council by Unglaub W G *et al* (J A M.A 161 623 June 16, 1956) is cited which states The amount of vitamin B_{12} required for maintenance therapy of patients with pernicious anemia is approximately 1 to 2 mcg daily The giving of 100 mcg intramuscularly at monthly intervals has been

found satisfactory for most patients The use of larger doses given at longer intervals does not seem advisable since the amount of vitamin excreted in the urine increases as the dosage is raised High oral doses of vitamin B_{12} (in milligrams rather than micrograms) are not recommended for routine use in pernicious anemia

23 Lowther C P Alexander W D and Hendry E B Oral treatment of perniciousanaemia Lancet 1 495-497, March 6 1954

Twenty patients with pernicious anemia in remission and controlled adequately with injections of vitamin B_{12} or purified liver extract have since been treated satisfactorily with an oral preparation which contains 1 5 Gm of hog pyloric mucosa and 7.5 mcg of vitamin B_{12} per tablet Two tablets daily were given to 10 patients the other 10 received four tablets Subacute degeneration of the cord was present in 3 patients 1 had active pulmonary tuberculosis and received streptomycin and PAS (para aminosalicylic acid) throughout the six months of the study There was no evidence of hematologic or neurologic relapse after six months continuous treatment

Western I firmary
Glasgow Scotland

24 Meulengracht E Treatment of pernicious anaemia with very small quantities of pyloric mucosa and vitamin B_{12} Brit M J 1 838 841, April 10 1954

A commercial preparation for oral use containing dried pyloric mucosa from pig stomach and vitamin B_{12} proved effective in pernicious anemia Initial dosage was 300 mg of dried mucosa and 30 mcg vitamin B_{12} Maintenance dose has not yet been determined The mucosa alone 100 or 200 mg three times daily had a definite but slight anti anemic effect in patients with untreated pernicious anemia When these or smaller dosages were given with 10 to 15 mcg of oral vitamin B_{12} there was a strong anti anemic effect

Bi pebjerg Hospital and
Biological Ch mistry Labo atories of
th Medicinalco
Copenhagen Denmark

25 Brücher H Oral treatment of pernicious anemia with a compound containing vitamin B_{12} and pyloric mucosa Deutsche med Wchnschr 79 1726-1728 Nov 12 1954 (abstr J A M A 157 545 546 Feb 5 1955)

Vitamin B_{12} 5 mcg and powdered pyloric mucosa 130 mg combined in an oral tablet were given to 27 pernicious anemia patients twice daily Bone marrow became normal in all patients

Stuttgart Germany

26 Bethell F H Castle W B Schwartz S O and Wintrobe M M I The use of intrinsic factor B_{12} combinations in the treatment of pernicious anemia Blood 10 377 379 April 1955 (in Panels in Therapy)

In a panel discussion on pernicious anemia treatment Dr F H Bethell stated that the more nearly physiologic method of providing vitamin B_{12} is by its daily oral administration together with intrinsic factor For this purpose only U S P official preparations to which U S P oral unitage has been assigned should be used Such therapy is suitable for intelligent dependable patients who do not have chronic intestinal disorders or severe neurologic damage Injections at stated intervals insure the patient s receiving the therapy and returning for periodic observation

Dr W B Castle preferred use of parenteral injection of vitamin B_{12} or Liver Injection U S P as it is more certain more convenient and less expensive than oral therapy Adequate parenteral maintenance therapy of pernicious anemia requires only that the patient receive 1 cc of Vitamin B_{12} Injection U S P containing 30 mcg of vitamin B_{12} at four week intervals

Objection to the routine use of intrinsic factor and vitamin B_{12} was made by Dr S O Schwartz on the basis that no long term studies substantiating hematologic control or prevention of neurologic complications are available He recommends using intrinsic factor and vitamin B_{12} only in cases of sensitization to parenteral liver and vitamin B_{12}

Since patients can be maintained in excellent remission by injections, Dr M M Wintrobe stated that it has not been necessary so far to use the intrinsic factor vitamin B_{12} combinations in the treatment of pernicious anemia He pointed out that danger of relapse due to the patient s omitting medication is great

Dr W Dameshek commenting as the moderator agreed with Dr Bethell that continued oral medication seems more distinctly physiologic since a small though definite amount of the deficient material is supplied daily Giving an injection even every month or two might be viewed as a distinctly unphysiologic means of supplying vitamin B_{12} In the patient with pernicious anemia in remission who can afford the procedure it has been his custom to give an injection of liver extract or vitamin B_{12} once every month or two or three and have him take a pill of one of the intrinsic factor vitamin B_{12} preparations every day

University of Michigan Medical School
Ann A bor Mich

Thorndike Memorial Laboratory City Hospital
Boston Mass

Cook County Hospital
Chicago Ill

Uni ersity of Utah Medical School
Salt Lake City Utah

27 Blackburn E K Cohen, H and Wilson G M Oral treatment of pernicious anaemia with a combined vitamin B_{12} and intrinsic factor preparation Brit M J 2 461-463, Aug 20 1955

Five patients with newly diagnosed addisonian pernicious anemia were treated initially with 30 mcg of crystalline vitamin B_{12} and 150 mg of intrinsic factor per day given orally Four patients showed satisfactory response and one who did not respond later improved on parenteral vitamin B_{12} A group of 12 patients who had had maintenance therapy with parenteral vitamin B_{12} for at least a year were then given 6 mcg of vitamin B_{12} and 30 mg of intrinsic factor daily for one year They were compared with 12 other patients of similar age and clinical state who were kept on parenteral vitamin B_{12} therapy During the second six months of the trial year the patients on the oral preparation showed declines in hemoglobin and red cell counts but the symptomatic and clinical state remained the same One patient not in the series above showed signs of subacute degeneration of the cord on maintenance therapy with the oral preparation but improved after injections of vitamin B_{12}

The authors believe that the variable activity of the intrinsic factor may account for the erratic response to the oral preparation They point out that oral maintenance therapy is at present much more expensive than parenteral Patients however generally preferred oral therapy

University of Sheffield and
Sheffield Royal Infirmary
Sheffield England

28 Conley C L and Krevans, J R New developments in the diagnosis and treatment of pernicious anemia Ann Int Med 43 758-766 Oct 1955

In pernicious anemia satisfactory remissions have been obtained using preparations containing vitamin B_{12} and intrinsic factor However, further extensive clinical trials are required to establish that these preparations are as reliable as is the parenteral injection of vitamin B_{12} Satisfactory responses can be obtained with very large amounts of vitamin B_{12} administered orally without intrinsic factor The authors have treated 45 patients with oral vitamin B_{12} alone The initial dose ranged from 3 000 mcg to 10 000 mcg In most cases the hematologic and

clinical response to these large oral doses equalled that obtained with 30 mcg of parenteral vitamin B_{12}. Remissions were sustained with oral doses of 1 000 mcg weekly

Measurements of vitamin B_{12} level in serum in 7 pernicious anemia patients maintained on oral vitamin B_{12} compared to those in patients receiving vitamin B_{12} intramuscularly and to those in normal subjects indicated that doses of 1 000 mcg orally are probably suboptimal and higher doses would be necessary for tissue saturation

It is emphasized that only a small percentage of orally administered vitamin B_{12} can be absorbed by pernicious anemia patients and their ability to absorb the vitamin varies The complete effectiveness of monthly parenteral therapy using much smaller doses indicates that it is the preferred method of administration for most patients with pernicious anemia particularly those with neurologic symptoms However if oral therapy is used crystalline vitamin B_{12} alone in milligram doses appears as effective as smaller amounts of the vitamin combined with intrinsic factor

Johns Hopkins University and Hospital Baltimore Md

29 Chalmers J N M and Hall Z M Treatment of pernicious anaemia with oral vitamin B_{12} without known source of intrinsic factor Brit M J 1 1179 1181, May 22 1954

Oral vitamin B_{12} in amounts ranging from 9 000 mcg in a single dose to repeated daily doses of 50 mcg was administered without known source of intrinsic factor to 12 patients with pernicious anemia in relapse and to 3 gastrectomized patients with similar symptoms Oral vitamin B_{12} alone proved to be hemopoietic when given either in large single doses of 2 000 mcg or more or in repeated daily doses of 50 mcg The uniformly good results obtained with daily oral doses of 50 mcg may be related to the fact that all patients took the dose when fasting and had no food for six hours afterward The investigators conclude Our results demonstrate again that extraneous sources of intrinsic factor are not essential for utilization of vitamin B_{12} and that oral therapy with the vitamin alone may be most effective when given to fasting patients

St George's Hospital and Medical School London England

30 Reisner E H Jr Weiner L Schittone M T and Henck E A Oral treatment of pernicious anemia with vitamin B_{12} without intrinsic factor New England J Med 253 502 506 Sept 22, 1955

For periods from 12 to 28 months 43 patients with pernicious anemia were maintained on oral vitamin B_{12} without intrinsic factor Doses of 1 mg vitamin B_{12} were given at intervals ranging from twice weekly to once every four weeks the majority receiving treatment at one or two week intervals

The authors state These studies indicated to our satisfaction that regular oral doses of 1 mg of vitamin B_{12} would maintain patients with pernicious anemia in satisfactory hematologic and neurologic remission when given at weekly intervals

New York University Post Graduate Medical School New York N Y

31 Estren S and Wasserman L R Pernicious anemia I Remission by small oral doses of purified vitamin B_{12} Proc Soc Exper Biol & Med 91 499 503 March 1956

Vitamin B_{12} 5 0 to 16 8 mcg was given orally one to three times daily without intrinsic factor to 9 patients with pernicious anemia in relapse and to 1 with megaloblastic anemia Maximal reticulocytosis (within 8 to 9 days) and hematologic response with reversion of bone marrow to normoblastic erythropoiesis occurred in 3 cases of pernicious anemia and 1 of megaloblastic anemia In

5 pernicious anemia cases response was submaximal in 1 none occurred Remission extended four to six months after therapy ended Two patients who responded initially failed to respond to this regimen when again in relapse

The authors state that the results support the suggestion that in pernicious anemia a quantitative rather than a qualitative change in gastric secretion occurs, and that varying amounts of intrinsic factor in pernicious anemia patients may cause varying responses to oral vitamin B_{12} A trial of vitamin B_{12} alone should precede trial of a vitamin B_{12} plus intrinsic factor preparation

Mount Sinai Hospital
New York N Y

32 **Monto R W and Rebuck, J W Observations on the mechanism of *intranasal absorption of vitamin B_{12}* in pernicious anemia, Blood 10 1151-1155, Nov 1955**

Vitamin B_{12} (without intrinsic factor) was administered intranasally to 32 patients with pernicious anemia in relapse and to 55 patients in remission The vitamin produced equal response when given in saline solution, in lactose powder, or when used as crystals Doses of 100 mcg brought about maximal reticulocyte response in the patients in relapse and provided adequate maintenance levels in patients in remission The hematologic gastrointestinal and neurologic improvement was comparable to that noted after parenteral therapy

Nasal washings taken from a normal subject after instillation of 150 mcg of vitamin B_{12} were administered to one patient by means of a gastric tube There was no significant increase in the reticulocyte count

Urinary bioassay for vitamin B_{12} performed in a patient who received 200 mcg of vitamin B_{12} intranasally showed that 12 per cent of the administered dose was excreted These studies suggest the direct absorption of the vitamin B_{12} through the respiratory mucosa rather than binding or combining with a mucoprotein (intrinsic factor) of the nasal secretions

Henry Ford Hospital
Detroit Mich

33 **Monto R W, Rebuck, J W and Howell, J T The single intra nasal application of vitamin B_{12} crystals in pernicious anemia A clinical and laboratory appraisal of two patients J Lab & Clin Med 44 900, Dec. 1954 (in Soc Proc)**

In a patient with an initial erythrocyte count of 2 2 millions 150 mcg of vitamin B_{12} crystals applied to the nasal mucosa caused reticulocytosis of 44 5 per cent on the 6th day Complete remission (hemoglobin 16 5 Gm red blood count 5 65 millions) was obtained for three months *without further treatment*

A patient in relapse with an erythrocyte count of 2 01 millions was given 200 mcg vitamin B_{12} crystals on the nasal mucous membrane A reticulocytosis of 43 4 per cent occurred on the 7th day A complete clinical remission was obtained with an increase of erythrocytes to 4 12 millions in a two-month period without further treatment.

It was concluded that a single application of vitamin B_{12} to the nasal mucosa effected complete clinical and laboratory response in these 2 pernicious anemia patients The nonirritating vitamin B_{12} crystals were rapidly absorbed by the nasal mucous membranes Absorption of the vitamin B_{12} by the nasal mucosa is probably not dependent upon binding with intrinsic factor

Detroit Mich

34 **Monto, R. W and Rebuck, J W Nasal instillation and inhalation of crystalline vitamin B_{12} in pernicious anemia, A M A Arch Int Med. 93 219 230, Feb 1954**

Since the success of parenteral injection of vitamin B_{12} is attributed to ready

access to blood and lymph capillaries and since one of the largest capillary beds in contact with the external environment is that of the pulmonary circulation inhalation administration of vitamin B_{12} seemed logical The similarity of the mucosa of the nose and the tracheobronchial tree suggested nasal instillation of this vitamin Concentrations of 15 to 200 mcg of vitamin B_{12} per cc of isotonic sodium chloride solution were used a concentration of 100 mcg per cc gave maximum hemopoietic effect For administration as dust, 1 000 mcg of vitamin B_{12} in 0 1 cc. by volume of lactose powder was used

Both inhalation and nasal instillation were used in treating 12 patients with pernicious anemia in relapse and 20 with pernicious anemia in remission No evidence of toxicity or sensitivity at the local site was observed A satisfactory hematologic and clinical response comparable to that expected from parenteral administration was obtained in the patients in relapse and the condition of patients in remission had been maintained for as long as 18 months at the time of writing

Henry Ford Hospital
Detroit Mich

35 Israels M. C G and Shubert S The treatment of pernicious anaemia by insufflation of vitamin B_{12} Lancet 1 341 343 Feb 13, 1954

Five patients with pernicious anemia were successfully treated with vitamin B_{12} in the form of snuff for insufflation Doses were of the same order as those commonly used for intramuscular administration

University of Manchester and
R yal I firmary
Manchester England

PERNICIOUS ANEMIA ANALYTICAL STUDIES

36 Hamilton H E DeGowin E L Sheets R. F Janney C D and Ellis J A. Studies with inagglutinable erythrocyte counts VI Accelerated destruction of normal adult erythrocytes in pernicious anemia contribution of hemolysis to the oligocythemia J Clin Investigation 33 191 205 Feb 1954

Transfusions of fresh normal erythrocytes to patients with pernicious anemia suggest that vitamin B_{12} deficiency adversely affects normal circulating adult erythrocytes Pretreatment with 15 mcg of vitamin B_{12} daily for 9 days appeared sufficient to prevent hemolysis of transfused erythrocytes but treatment for 3 days was apparently insufficient Daily administration of 15 mcg of vitamin B_{12} for 23 days protected transfused erythrocytes from random destruction for only 20 days *after cessation of therapy*

In 4 of 6 patients with untreated pernicious anemia the oligocythemia could be entirely accounted for by random erythrocyte destruction as measured by the loss of normal transfused cells In 2 other patients maturation arrest in the bone marrow was a factor in the oligocythemia but part of it could be attributed to abnormally rapid destruction of erythrocytes

State Uni ersity of Iowa
Iowa City Iowa

37 Glazer H S Mueller J F Jarrold T Sakurai K Will J J and Vilter R W The effect of vitamin B_{12} and folic acid on nucleic acid composition of the bone marrow of patients with megaloblastic anemia J Lab & Clin Med 43 905 913 June 1954

Ribonucleic and desoxyribonucleic acid (RNA and DNA) thymine (T) and uracil (U) concentrations in the bone marrow of 11 patients with pernicious anemia and in 1 with sprue were determined by paper chromatography before during and after treatment with one or more of the following ascorbic acid vitamin B_{12} folic acid and folinic acid Similar determinations were made on 19 normal subjects

The RNA/DNA and U/T ratios were higher than normal in patients with meg

aloblastic anemia, the relative amount of thymine was lower than normal After adequate treatment with hemopoietic vitamins the RNA/DNA and U/T ratios fell and the relative amount of thymine rose to normal as the bone marrow became normal cytologically

The results suggest that in hemopoietic tissues, vitamin B_{12} folic acid and ascorbic acid promote formation of thymine and its desoxyribotides and depletion of uracil and its nucleotides There is also evidence that a greater percentage change takes place in the pyrimidine concentrations than in the nucleic acid concentrations This may indicate that a *qualitative effect on the composition of* the nucleic acids is initiated by these vitamins

The following daily dosages were employed (1) ascorbic acid orally 1,000 mg (2) vitamin B_{12} intramuscularly, 0 25 mcg or 5 to 30 mcg (3) folic acid intramuscularly 0 5 or 10 mg (4) folinic acid intramuscularly, 1 mg

University of Cincinnati
College of Medicine
Cincinnati Ohio

38 Sandberg A A Eik Nes K Nelson D H, Palmer, J G Cartwright, G E and Wintrobe, M M Adrenocortical function and metabolism of 17 hydroxycorticosteroids in pernicious anemia New England J Med 251 169 174 July 29 1954

Adrenocortical function and metabolism of 17 hydroxycorticosteroids in pernicious anemia were studied by measurement of the blood 17 hydroxycorticoids in 8 patients with pernicious anemia in relapse and after vitamin B_{12} therapy in normal controls and in 1 patient with achylia gastrica

There was no evidence that adrenocortical function or steroid metabolism is impaired in patients with pernicious anemia in relapse Normal 17 hydroxycorticoid levels were found during relapse (except in 2 severely ill patients whose 17 hydroxycorticoid levels were elevated) and after vitamin B_{12} therapy The adrenal cortex responded normally to corticotropin administration One patient with *pernicious anemia in relapse* was given hydrocortisone intravenously and 2 were given cortisone intravenously The rate of disappearance of 17 hydroxycorticosteroids from the plasma was normal

After oral administration of 200 mg of hydrocortisone acetate suspension to 4 patients with pernicious anemia and 200 mg of hydorcortisone suspension to 1 patient with pernicious anemia plasma 17 hydroxycorticoid levels were elevated for prolonged periods The magnitude of the elevation was not significantly influenced *by vitamin* B_{12}

A patient with achylia gastrica and no evidence of anemia showed the same prolonged 17 hydroxycorticosteroid level after oral hydrocortisone as the patients with pernicious anemia Ten patients with pernicious anemia were given 200 mg of hydrocortisone suspended in 150 cc of normal gastric juice The 17 hydroxycorticosteroids were lower than when hydrocortisone was given without gastric juice

The data derived from the study of the patient with achylia gastrica without *pernicious anemia* indicate that the presence of achylia per se rather than its metabolic effects, is the important factor in the response Failure of vitamin B_{12} therapy to modify the results further implicates the achylia

Salt Lake City Utah

39 Pollycove M, Apt, L and Ross J F Erythropoiesis and the movement of iron in patients with pernicious anemia Clin Research Proc 3 92 93, April 1955

The movement *of iron in* 7 patients with pernicious anemia before and after initial therapy with vitamin B_{12} was studied with the use of tracer doses of Fe^{59} Before treatment the rate of removal of iron from the plasma was 4 to 6 times normal only 10 to 20 per cent was incorporated into circulating erythrocytes (*nor*

mal 90 to 100 per cent) and the iron initially accumulated with abnormal rapidity in the bone marrow and spleen After retention in the marrow for several days most of the iron gradually moved to the liver

Three days after the initiation of vitamin B_{12} therapy radioactive iron was rapidly depleted from the liver and bone marrow A simultaneous sharp increase of radioactive iron within circulating erythrocytes occurred

These studies demonstrate a slight destruction of circulating erythrocytes but emphasize the predominance of arrested erythrocyte hemoglobinization in producing pernicious anemia They also suggest that the abnormal increment of fecal urobilinogen in this disease is probably derived from the breakdown of heme or other porphyrins within the bone marrow

Boston V A Hospital and
Tufts College Medical School
Boston, Mass

40 **Rabiner S F and Lichtnam H C. Studies on the relationship between the peripheral blood counts and the total body red cell volume A M A Arch Int Med 99 474–480 March 1957**

It has been shown previously that in normal persons there is a relatively constant relationship between the hematocrit of peripheral venous blood and the circulating red blood cell volume per kilogram of body weight (CRBCV/kg)

Thirteen patients with Addisonian pernicious anemia were studied by determining the circulating red blood cell volume using isotopically tagged red blood cells

A poor correlation between the peripheral venous hematocrit and the circulating red blood cell volume per kilogram body weight was found in patients with pernicious anemia in relapse and during regeneration In some instances the venous hematocrit was falsely high and other times falsely low In a few selected cases this poor relationship was also found in patients with Addisonian pernicious anemia during remission

The data indicate that the degree of blood regeneration in response to specific therapy cannot always be estimated by the changes in peripheral blood cell counts The hematologic response as determined by changes in CRBCV/kg of body weight may be greater than that indicated by the changes noted in the venous blood In some persons the clinical state seems to be as useful a guide in evaluating the response to therapy as is the estimation of the red blood cell volume

St te Uni ersity of
New York College of Medicine
New York N Y

41 **Thomas E D and Lochte H L Jr In vitro observations on oxygen consumption heme synthesis and desoxyribonucleic acid synthesis by pernicious anemia bone marrow Clin Research Proc 5 145 146 April 1957**

The nature of the biochemical defect in pernicious anemia (P A) is still not understood We have studied P A bone marrow by the following technics (1) Oxygen consumption (2) Heme synthesis as measured by the rate of incorporation of C^{14} glycine into heme and (3) Desoxyribonucleic acid (DNA) synthesis by measurement of the rate of incorporation of C^{14} formate into thymine

Utilizing these methods it was found that P A bone marrow synthesizes INA at a greater rate in normal serum than in P A serum Addition of vitamin B_{12} to P A bone marrow in P.A serum accelerates the rate of DNA synthesis B_{12} and folic acid have no effect on oxygen consumption or heme synthesis Folic acid and citrovorum factor accelerate markedly DNA synthesis in some marrow samples and show no effect in others P A serum has no demonstrable inhibitory effect on normal bone marrow From these studies it is concluded that vitamin B_{12} and folic acid are specifically con

cerned with DNA synthesis that B_{12} has a direct action on the marrow cells and that folic acid affects some P A marrows but not others

These studies provide evidence of the importance of B_{12} in DNA synthesis by human cells The technics described make it possible to apply the data from numerous microbiologic studies to human cells

Mary Imogene Bassett Hospita
Cooperstown N Y

NUTRITIONAL MACROCYTIC ANEMIAS

42 Das Gupta C R., Chatterjea, J B and Basu P Vitamin B_{12} in nutritional macrocytic anaemia, Brit M J 2 645 649, Sept 19 1953

In a Calcutta hospital 22 Indian patients with uncomplicated nutritional macrocytic anemia were treated with vitamin B_{12} Fifteen received intramuscular therapy 1 received oral therapy and 6 received both at different times In general maximum response followed 200 to 300 mcg of intramuscular vitamin B_{12} but dose and response had no distinct correlation There was no correlation between response to oral vitamin B_{12} with either dose or state of gastric acidity Initial improvement occurred in 14 cases but was sustained in only 5 Reticulocytosis was inadequate and was not correlated with the degree of improvement Folic acid and crude liver extract given to patients who relapsed gave adequate or better results in most cases

The authors state that these studies definitely indicate that nutritional macrocytic anemia involves deficiency of both folic acid and vitamin B_{12} Deficiency of folic acid however is described as the major and primary factor and that of vitamin B_{12} the minor and secondary factor in most cases

School of Tropical Medicine
Calcutta India

43 Stahlie, T D Megaloblastic anemia and vitamin B_{12}, Maandschr v kindergeneesk 21 396, Dec 1953 (abstr J A M A 154 1137, March 27, 1954)

A child with megaloblastic anemia recovered after treatment with intramuscular vitamin B_{12} followed by oral vitamin B_{12} The daily dosage by both routes was 5 mcg Maintenance therapy with vitamin B_{12} was not necessary It is believed that nutritional deficiencies during the war were responsible for the anemia

44 Watson, R J, Lichtman, H C Messite J, Ellison, R R, Conrad, H and Ginsberg V Clinical studies with the citrovorum factor in megaloblastic anemia Am J Med 17 17 28 July 1954

Five patients with nutritional megaloblastic anemia had hematologic remissions after therapy with citrovorum factor (CF) Four had previously failed to improve when given vitamin B_{12} Two patients apparently had dual deficiencies of both vitamin B_{12} and CF One had addisonian pernicious anemia and a superimposed deficiency of CF the other had cirrhosis of the liver and a nutritional megaloblastic anemia The latter showed a good hematologic remission initially when given vitamin B_{12} but subsequently relapsed and responded only to the administration of CF

Treatment of a patient with addisonian pernicious anemia with CF resulted in acute combined system disease which cleared up promptly after parenteral administration of vitamin B_{12} The use of CF or PGA (pteroylglutamic acid) is undoubtedly contraindicated in addisonian pernicious anemia It is possible that the hematologic response to these agents may further deplete the stores of vitamin B_{12} accelerating the development of combined system disease In megaloblastic anemias with associated gastric achlorhydria vitamin B_{12} therefore is the drug of choice When hydrochloric acid is found

in the gastric juice treatment with CF or PGA is indicated

State University of New York
New York N Y
Kings County Hospital
Brooklyn N Y

45 Gosselin B and Morissette L. Le *traitement des anémies macrocytaires* par la vitamine B_{12} orale [Treatment of macrocytic anemias by oral vitamin B_{12}] Union méd du Canada 83 1238-1241, Nov 1954

A patient with nutritional macrocytic megaloblastic anemia was successfully treated with a preparation which contains per capsule 100 mcg of vitamin B_{12} 75 mg of extract of the duodenal mucosa and 1 75 mg of folic acid At the time the case was reported the patient had resumed his work and was being maintained in perfect health by a vitamin B_{12} preparation

Despite the efficacy of this oral preparation, the authors are not convinced that oral treatment is superior in general to intramuscular administration since an injection every two or three weeks maintains the patient in good condition When injections are not feasible however oral treatment will render great service

Hôpital Notre-Dame
Montreal Canada

46 Pollycove, M Apt L and Colbert M. J Pernicious anemia due to dietary deficiency of vitamin B_{12} Clin Research Proc. 3 28 Feb 1955

A man of 60 who had scrupulously avoided all foods of animal origin for at least 8 years was admitted with a severe macrocytic anemia associated with a megaloblastic bone marrow The serum vitamin B_{12} level was 70 micromicrograms per cc and hematologic response to parenterally administered vitamin B_{12} was characteristic The patient s gastric juice contained free hydrochloride and when it was administered with Co^{60} vitamin B_{12} to a patient with pernicious anemia normal absorption resulted as indicated by low fecal elimination and a high hepatic uptake The patient himself exhibited normal absorption of Co^{60} vitamin B_{12} after remission of the anemia Thus this patient in spite of adequate intrinsic factor apparently developed vitamin B_{12} deficiency as the result of a prolonged dietary deficiency of it

Tufts College Medical School and
Boston V A Hospital
Boston Mass

47 Wokes F, Badenoch, J and Sinclair H M Human dietary deficiency of vitamin B_{12} Am J Clin Nutrition 3 375 382 Sept Oct 1955

The authors compare the dietary intake of calories protein and vitamin B_{12} and describe vitamin B_{12} deficiency symptoms in American Dutch and British vegans (vegetarians who do not eat dairy products) The commonest and earliest symptoms of vitamin B_{12} deficiency were oral Paresthesia developed more slowly Amenorrhea and menstrual disorders occurred in 8 of 22 British women Nervous symptoms were common as were *pains and stiffening of the spine* and back The incidence of deficiency symptoms was lower in the Dutch than in the British and did not appear among the 26 Americans all of whom had been vegans five years or longer Serum vitamin B_{12} levels showed a steady fall during the first 7 years a return to near normal between 7 and 10 years and then a more gradual descent In vegans with anemia correlation of the red cell counts with vitamin B_{12} levels gave an average curve unlike that seen in pernicious anemia but similar to that seen in subacute combined degeneration of the cord This may reflect the large ingestion of folic acid

The nervous system symptoms observed in some vegans might have been due to chronic toxicity caused by an increase in the blood concentration of a keto acid derivative of tyrosine Urinary excretion and blood levels of thiocyanates

were also increased and were further stimulated after tyrosine ingestion

The results of the investigation indicate the occurrence in man of a dietary deficiency of vitamin B_{12} similar to that encountered in animals living on a diet free from animal protein The poor rate of growth and development in such animals can be improved by addition of vitamin B_{12} provided that their diet is not deficient in total protein

The authors attribute the varying incidence of deficiency symptoms in the Dutch British and Americans to the variations in total protein intake in the three groups The British who had the lowest intake (7 per cent) had the highest incidence of deficiency while the Americans who ingested the largest amount of protein (10 4 per cent) had no clinical deficiency symptoms

Ovaltine Research Laboratories
King s Langley England
Radcliffe Infirmary and
University of Oxford
Oxford England

ANEMIAS OF PREGNANCY

48 Das Gupta C R , Chatterjea J B and Basu P Vitamin B_{12} in macrocytic anemia in pregnancy Indian M Gaz 88 102 105 1953 (abstr Blood 10 667 668 June 1955)

Twenty one cases of macrocytic anemia in pregnancy 13 with megaloblastic and 8 with normoblastic bone marrow *were treated with vitamin B_{12} by the oral* or the intramuscular route In both groups the response was better by the intramuscular route Initial response was good in 70 per cent of cases with megaloblastic marrow and in 80 per cent of cases with normoblastic marrow The good initial response was not however sustained and continuation of vitamin B_{12} therapy did not usually produce further improvement of blood picture but administration of folic acid or crude liver extract did The authors suggest that an average case of macrocytic anemia in pregnancy represents combined deficiency of folic acid and vitamin B_{12}

School of Tropical Medicine
Calcutta India

49 Clarke, J P and Essig, L L Megaloblastic anemia of pregnancy, Am J Obst & Gynec 67 367 372, Feb 1954

Case histories of 3 patients with megaloblastic anemia of pregnancy are presented The first patient received intramuscular vitamin B_{12} (30 mcg daily) for two days prior to delivery Vitamin B_{12} rather than folic acid was given in this case because of lack of appreciation of the greater specificity of folic acid in this condition The authors describe the response to vitamin B_{12} as inadequate The other 2 patients were treated with folic acid 10 mg and 15 mg by mouth daily respectively Both showed an adequate reticulocyte and clinical response

University of Colorado
School of Medicine
Denver Colo

50 Moore H C Lillie, E H and Gatenby, P B B The response of megaloblastic anaemia of pregnancy to vitamin B_{12} Irish J M Sc 6th Series No 351 106, March 1955 (abstr Am J Obst & Gynec 71 1163, May 1956)

Seventeen cases of megaloblastic anemia of pregnancy at the Rotunda Hospital were treated with vitamin B_{12} Six patients also received iron therapy *Fecal fat analysis* was performed during a four-day period with the patients on a standard diet containing 70 grams of fat and 70 grams of protein daily Thirteen patients had a reticulocyte response to therapy The four cases which did not respond to vitamin B_{12} therapy responded favorably to subsequent folic acid therapy The authors conclude that there are two groups of megaloblastic anemia of pregnancy depending on the response to vitamin B_{12} and not all cases can be regarded as folic acid deficient anemias

51 Badenoch J Callender S T Evans J R Turnbull A L and Witts L J Megaloblastic anemia of pregnancy and the puerperium Brit M J 1 1245-1247 May 21 1955

In 9 patients with megaloblastic anemia of pregnancy and the puerperium intrinsic factor secretion fat absorption and serum vitamin B_{12} levels were normal Of the 4 examined before delivery 2 responded to folic acid and 2 did not respond either to folic acid or to vitamin B_{12} therapy The 3 patients studied during the puerperium seemed to respond to folic acid or to vitamin B_{12} but these recoveries might have been spontaneous remissions which are common after delivery Of 2 patients with severe anemia six weeks after delivery 1 responded to folic acid and 1 to vitamin B_{12}

The authors attribute the variable findings to toxemia and infection, hemorrhage multiple or rapidly repeated pregnancies or to poor diet and gastrointestinal upsets However when pregnancy alone seems to induce anemia the cause appears to be the development of resistance to vitamin B_{12} and folic acid

Radcliffe Infirmary
Oxford England

52. Berry C G Anaemia of pregnancy in Africans of Lagos Brit M J 2 819-823 Oct 1 1955

A study of the anemia of pregnancy among African women in Lagos included 30 patients The diet of these people consists mainly of starchy vegetables palm and groundnut oil with little meat eggs milk or fruit The red cell counts in the 30 patients ranged from 0 69 million to 3 4 million per cu mm The hemoglobin concentration ranged from 2 5 to 11 Gm per 100 cc Leukocyte counts ranged from 4 300 to 19 600 per cu mm Stool examinations showed worm infestations in many of the patients which undoubtedly aggravated the anemia

Vitamin B_{12} 100 or 200 mcg initially followed by 20 or 50 mcg at weekly intervals was administered to 13 patients Of the 9 patients treated during pregnancy 4 responded rapidly and 3 more slowly Clinical improvement in all these was striking Of the 4 patients treated after delivery 2 showed no response to vitamin B_{12} or any other agent and 2 patients responded well However this response could not be attributed completely to vitamin B_{12} because of the possibility of spontaneous remission following delivery

The author states that although the primary deficiency among these women may have been folic acid response to folic acid may not be possible until the secondary deficiency of vitamin B_{12} is corrected After parturition the slow spontaneous recovery which occurs as folic acid again becomes available may be accelerated by administration of vitamin B_{12}

St James' Hospital
London England

53 Lowenstein L Pick C and Philpott, N Megaloblastic anemia of pregnancy and the puerperium Am J Obst & Gynec 70 1309 1337, Dec 1955

In 18 patients with anemia of pregnancy or the puerperium dietary deficiency was the apparent cause in 12 causes in the other 6 were not definitely known Treatment consisted of oral or intramuscular vitamin B_{12} or folic acid or oral folinic acid Many of the patients received more than one of these agents in sequence

Vitamin B_{12} was effective by the oral route in 1 patient and by the parenteral route in 3 It failed in 4 patients who were later successfully treated with folic acid A complete response was obtained with folic acid alone in 1 patient Folinic acid produced good response in 6 patients while 3 had spontaneous remissions

In June 1953 the authors began routinely prescribing prenatal supplements which included vitamin B_{12} and folic acid They believe that this is responsible for

the fact that since then no patients with megaloblastic anemia of pregnancy have been admitted to the hospital

It is concluded that in temperate climates megaloblastic anemia associated with pregnancy or the puerperium may result from a deficiency of folic acid or vitamin B_{12} or possibly of a third unknown factor

McGill Medical School and
Royal Victoria Hospital
Montreal Quebec

54 Adams, E B Treatment of megaloblastic anaemia of pregnancy and the puerperium with vitamin B_{12}, Brit M J 2 398-400 Aug 18, 1956

Author s summary The results of a clinical trial using vitamin B_{12} in 10 patients with megaloblastic anaemia of pregnancy are recorded The appearance of the bone marrow is briefly described

Response was satisfactory in all seven treated after delivery It appeared to be as good with doses of 100 µg as it was when 1 000 µg or more was given, but better results are usually obtained with folic acid

Three patients treated before delivery responded poorly

Because of differences in the appearance of the bone marrow and in response to treatment it seems likely that there are several varieties of megaloblastic anaemia associated with pregnancy

University of Natal
Durban South Africa

55 Izak G Rachmilewitz M Stein Y, Berkovici, B, Sadovsky, A Aronovitch, J and Grossowicz N Vitamin B_{12} and iron deficiencies in anemia of pregnancy and puerperium A M A Arch Int Med 99 346-355 March 1957

Of 2 500 women examined during pregnancy or the puerperium 276 were anemic The group was generally malnourished

Repeated hematologic examinations and serum vitamin B_{12} determinations were carried out in 100 of the anemic women Of these 30 had hypochromic iron deficiency anemia with low serum iron and normal serum vitamin B_{12} levels In 11 patients with hyperchromic macrocytic anemia very low vitamin B_{12} concentrations were found with normal or high serum iron concentrations The remaining 59 had dimorphic anemia, with both low serum vitamin B_{12} and low iron concentrations

A gradual fall to below 200 micromicrograms of the serum vitamin B_{12} concentration took place in most anemic patients during the last trimester of pregnancy, followed by a rise during the two to four weeks following delivery Vitamin B_{12} deficiency was attributable not only to the utilization of large amounts of the vitamin in the growth of the embryo but also to the fact that the diet during pregnancy as well as during preceding years had been poor in animal proteins

Though it has been generally accepted that megaloblastic anemia of pregnancy responds to crude liver extract and to folic acid but not to vitamin B_{12} recent reports have cited favorable therapeutic results in some patients treated with vitamin B_{12}

Hebrew University Hadassah Medical School
Jerusalem Israel

56 Thompson R. B Seasonal incidence of megaloblastic anaemia of pregnancy and the puerperium Lancet 1 1171 1172 June 8, 1957 (In Original Articles)

The author suggests that dietary deficiency is a significant factor in megaloblastic anemia of pregnancy in the tropics If this is true more patients may be expected to develop the disorder during winter and spring A study of 100 cases of megaloblastic anemia of pregnancy showed just such an increased incidence in winter and spring

Because much of the supply of folic acid comes from green vegetables it may be that dietary sources in the winter are

inadequate to meet the increased needs of pregnancy Vomiting of pregnancy further decreases intake The reason why patients with the disorder in temperate climates develop a predominantly if not exclusively folic acid deficiency is explained by Vinke and Van der Sar (1956) who point out that healthy people have a store of vitamin B_{12} which can supply their requirements for many months and sometimes for years The store of folic acid on the other hand can supply the needs of the body for only about a month In a previously well nourished person with increased demands for both substances and with a poor dietary intake folic acid deficiency becomes manifest much earlier than does a deficiency of vitamin B_{12} In a previously malnourished person with a low store of vitamin B_{12} however the deficiency is likely to be of both vitamin B_{12} and folic acid this would obtain in patients developing megaloblastic anaemia in the tropics

'The evidence presented here does not warrant the assumption that all cases of megaloblastic anaemia of pregnancy are due to dietary deficiency but it suggests that dietary deficiency is at least an important contributory cause It is interesting that the disorder disappeared from the wards of a maternity hospital in Montreal when an antepartum daily supplement of 4 5 µg of vitamin B_{12} and 3 0 mg of folic acid was given (Lowenstein *et al* 1955)

King's College University of Du ham and Princess Mary Maternity H spital Newcastle upo Tyne England

ANEMIAS OF INFANCY

57 **Cartagenova L So-called perniciosiform anemia with megalocytes of children, Lattante 24 856 Nov 1953 (abstr A M A. Am J Dis Child 88 689 Nov 1954)**

Perniciosiform anemia with megalocytemia was diagnosed in a boy of 13 months and a girl of 12 months who had hemolytic anemia megalocytes in the peripheral blood megaloblastic bone marrow glossitis and gastrointestinal disturbances Favorable results followed treatment with liver extract and vitamin B_{12} Aside from a few cases described in America practically all cases previously reported have been from Sicily These children were not Sicilian

58 **Roberto B Pernicious like anemia and other megaloblastic anemias of infancy Acta haematol 11 355 371, 1954 (abstr Blood 9 1122, Nov 1954)**

The author discusses the various megaloblastic anemias in infancy He stresses the curable types including pernicious like anemia of Gerbasi It is characterized by symptoms referable to the nervous system and by a response to small amounts of vitamin B_{12} given orally The disease seems to be due to insufficient vitamin B_{12} in the food

Uni ersity of Palermo Palermo Sicily Italy

OTHER ANEMIAS

59 **Kenawy, M R and Hassanein M A Combined vitamin B_{12} and folic acid therapy in iron deficiency anaemias Acta haemat 10 92 94 1953 (abstr Blood 9 296 March 1954)**

Experiments were carried out in cases of hypochromic anemia using a mixture of 30 mcg B_{12} and 10 mg folic acid for daily injections The average daily increase of hemoglobin in the cases treated with the vitamin B_{12} and folic acid in addition to the iron therapy was 1 77 per cent and 0 78 per cent in the control group with iron treatment alone

Kasr El Aini Fouad I Uni ity Cairo Egypt

60 Foy, H and Kondi, A Nutritional and intestinal factors and iron losses in the genesis of tropical anaemias, Lancet 1 423-424, April 14, 1956

The etiology and treatment of hypochromic, microcytic iron-deficiency anemias occurring in the tropics are discussed In the absence of iron deficiency such anemias might be due to intestinal blood loss caused by parasites defective intestinal iron absorption caused by abnormal calcium phosphorus ratios, or loss of iron through feces urine or sweat which is estimated to be 1 0 to 1 5 mg per day

Most patients with hypochromic microcytic iron deficiency anemias responded to the daily oral administration of iron or to iron and protein in some form Patients who did not respond to this treatment and who had giant stab cells in the urine often responded to 5 mg of folic acid daily for one week or 80 mcg of vitamin B_{12} or both

61 Foy H and Kondi, A Genesis of tropical anaemias, Lancet 2 95 96, July 14, 1956 (Letter to Editor)

Microcytic, hypochromic iron-deficiency anemia usually responds to iron but in some cases protein in the form of skimmed milk must also be given A smaller group of these anemias responds fully only if the patient is given in addition to iron and protein 80 to 100 mcg of intramuscular vitamin B_{12} in spite of the fact that the vitamin B_{12} serum level is normal Perhaps the reason is that in these anemias the vitamin B_{12} level in the serum must be higher to facilitate protein utilization and metabolism or for the synthesis of methyl and SH groups for hemopoietic purposes

Wellcome Trust Research Laboratories
Nairobi Kenya East Africa

NERVOUS SYSTEM

NEUROPATHIES DUE TO VITAMIN B_{12} DEFICIENCY

62 Bastrup Madsen P Non anaemic neuropathy caused by deficiency of the anti pernicious anaemia principle following subtotal gastrectomy Acta med Scandinav 147 399-404 Jan 29, 1954

Paresthesia occurred intermittently for 9 and 12 months after subtotal gastrectomy in 2 patients One patient also had muscle incoordination and ataxia The neurologic manifestations were similar to those accompanying pernicious anemia but there were no significant hematologic signs In both patients the symptoms disappeared after intramuscular administration of vitamin B_{12} and improvement continued during observation periods of 6 and 12 months

It is concluded that vitamin B_{12} deficiency may cause neuropathy without hematologic changes In this case subtotal gastrectomy may have caused the deficiency

Marselisborg Hospital
Aarhus Denmark

63 Jewesbury E C O Subacute combined degeneration of the cord and achlorhydric peripheral neuropathies without anaemia Lancet 2 307 312, Aug 14, 1954

Subacute combined degeneration of the cord occurred in 5 patients who showed only insignificant changes in the peripheral blood and who had normoblastic marrow All had histamine fast achlorhydria

In one case in which there was no initial treatment with vitamin B_{12} pernicious anemia developed one year later and terminated fatally In another case a small amount of vitamin B_{12} was given

for only two weeks Thereafter the patient remained well for three years but then developed pernicious anemia

In the 3 other patients who received adequate parenteral treatment with vitamin B_{12} pernicious anemia did not develop neurologic signs did not advance and there was symptomatic recovery In 2 of these 3 cases giant metamyelocytes were seen in the marrow this may be one of the earliest signs of pernicious anemia

Two other patients are described in whom peripheral neuropathy was associated with histamine fast achlorhydria and a normal peripheral blood count In 1 of these, minimal changes in the sternal marrow also suggested the beginning of pernicious anemia

Patients with histamine fast achlorhydria are liable to develop vitamin B_{12} deficiency but the relative susceptibility of the peripheral nerves spinal cord and marrow is unpredictable Early treatment and close observation of patients with neurologic manifestations is essential A few cases of nonanemic peripheral neuropathy with histamine fast achlorhydria may be due to causes other than vitamin B_{12} deficiency

The author suggests that the terms pernicious anemia and subacute combined degeneration of the cord be dropped in favor of the more comprehensive term vitamin B_{12} deficiency This might be qualified by adding megaloblastic anemia and/or neuropathy (cerebral spinal or peripheral)

Royal N rthern Hospital and North Middlesex Hospital London England

Chase Farm Hospital Enfield England

Luton and Dunstable Hospital Luton England

64 Arias I M Apt L and Pollycove M Absorption of radioactive vitamin B_{12} in nonanemic patients with combined-system disease New England J Med 253 1005 1010 Dec 8 1955

When hematologic manifestations of pernicious anemia do not occur it is difficult to differentiate combined system disease resulting from vitamin B_{12} deficiency from other types of neurologic disorders The authors describe a method for measuring gastrointestinal absorption of radioactive vitamin B_{12} which they used to make this differential diagnosis in 4 patients

Radioactive vitamin B_{12} was given orally with and without normal human gastric juice and also subcutaneously Scintillation from the liver was measured 10 days later The hepatic count after subcutaneous administration was assumed to represent 100 per cent absorption In the 4 subjects the hepatic count per minute per microcurie after oral dosage was 0 to 19 per cent of that seen after the subcutaneous dose When the same oral dose was given with normal human gastric juice the hepatic count was 59 to 63 per cent of that obtained after injection Gastric juice from the patients was given orally with radioactive vitamin B_{12} to other patients with treated pernicious anemia These patients were also given test doses of vitamin B_{12} without gastric juice subcutaneous doses and oral test doses with normal human gastric juice Hepatic counts were low when the radioactive vitamin or the vitamin plus gastric juice from the patients with combined system disease was given Counts after oral ingestion of the vitamin plus normal gastric juice were high When 11 normal subjects were tested hepatic deposition of orally administered radioactive vitamin B_{12} was 40 to 68 per cent of the amount noted after subcutaneous administration

Each of the 4 patients showed neurologic improvement on vitamin B_{12} therapy During follow up periods of from 2 to 96 months neurologic symptoms did not progress

Boston Uni er ity School of M di ine
T fts Uni ersity School of Medici e and
B ston Vete ans Administration Hospit l
Boston Mass

60 Foy, H and Kondi, A Nutritional and intestinal factors and iron losses in the genesis of tropical anaemias Lancet 1 423-424 April 14 1956

The etiology and treatment of hypochromic microcytic iron-deficiency anemias occurring in the tropics are discussed In the absence of iron deficiency such anemias might be due to intestinal blood loss caused by parasites defective intestinal iron absorption caused by abnormal calcium phosphorus ratios or loss of iron through feces urine, or sweat which is estimated to be 1 0 to 1 5 mg per day

Most patients with hypochromic microcytic iron deficiency anemias responded to the daily oral administration of iron or to iron and protein in some form Patients who did not respond to this treatment and who had giant stab cells in the urine often responded to 5 mg of folic acid daily for one week or 80 mcg of vitamin B_{12} or both

61 Foy, H and Kondi A Genesis of tropical anaemias, Lancet 2 95 96 July 14 1956 (Letter to Editor)

Microcytic hypochromic iron-deficiency anemia usually responds to iron, but in some cases protein in the form of skimmed milk must also be given A smaller group of these anemias responds fully only if the patient is given, in addition to iron and protein 80 to 100 mcg of intramuscular vitamin B_{12} in spite of the fact that the vitamin B_{12} serum level is normal Perhaps the reason is that in these anemias the vitamin B_{12} level in the serum must be higher to facilitate protein utilization and metabolism or for the synthesis of methyl and SH groups for hemopoietic purposes

Wellcome Trust Research Laboratories
Nairobi Kenya East Africa

NERVOUS SYSTEM

NEUROPATHIES DUE TO VITAMIN B_{12} DEFICIENCY

62 Bastrup-Madsen, P Non anaemic neuropathy caused by deficiency of the anti pernicious anaemia principle following subtotal gastrectomy, Acta med Scandinav 147 399-404 Jan 29 1954

Paresthesia occurred intermittently for 9 and 12 months after subtotal gastrectomy in 2 patients One patient also had muscle incoordination and ataxia The neurologic manifestations were similar to those accompanying pernicious anemia but there were no significant hematologic signs In both patients the symptoms disappeared after intramuscular administration of vitamin B_{12} and improvement continued during observation periods of 6 and 12 months

It is concluded that vitamin B_{12} deficiency may cause neuropathy without hematologic changes In this case subtotal gastrectomy may have caused the deficiency

Marselisborg Hospital
Aarhus Denmark

63 Jewesbury E C O Subacute combined degeneration of the cord and achlorhydric peripheral neuropathies without anaemia Lancet 2 307 312, Aug 14, 1954

Subacute combined degeneration of the cord occurred in 5 patients who showed only insignificant changes in the peripheral blood and who had normoblastic marrow All had histamine fast achlorhydria

In one case in which there was no initial treatment with vitamin B_{12} pernicious anemia developed one year later and terminated fatally In another case a small amount of vitamin B_{12} was given

for only two weeks Thereafter the patient remained well for three years but then developed pernicious anemia

In the 3 other patients who received adequate parenteral treatment with vitamin B_{12} pernicious anemia did not develop neurologic signs did not advance and there was symptomatic recovery In 2 of these 3 cases giant metamyelocytes were seen in the marrow this may be one of the earliest signs of pernicious anemia

Two other patients are described in whom peripheral neuropathy was associated with histamine fast achlorhydria and a normal peripheral blood count In 1 of these, minimal changes in the sternal marrow also suggested the beginning of pernicious anemia

Patients with histamine fast achlorhydria are liable to develop vitamin B_{12} deficiency but the relative susceptibility of the peripheral nerves spinal cord and marrow is unpredictable Early treatment and close observation of patients with neurologic manifestations is essential A few cases of nonanemic peripheral neuropathy with histamine fast achlorhydria may be due to causes other than vitamin B_{12} deficiency

The author suggests that the terms pernicious anemia and subacute combined degeneration of the cord be dropped in favor of the more comprehensive term vitamin B_{12} deficiency This might be qualified by adding megaloblastic anemia and/or neuropathy (cerebral spinal or peripheral)

Royal N rthern Hospital and
North Middlesex Hospital
London England

Chase Farm Hospital
Enfield England

Luton and Dunstable Hospit l
Luton England

64 Arias I M Apt L and Pollycove M Absorption of radioactive vitamin B_{12} in nonanemic patients with combined-system disease New England J Med 253 1005-1010 Dec 8 1955

When hematologic manifestations of pernicious anemia do not occur it is difficult to differentiate combined system disease resulting from vitamin B_{12} deficiency from other types of neurologic disorders The authors describe a method for measuring gastrointestinal absorption of radioactive vitamin B_{12} which they used to make this differential diagnosis in 4 patients

Radioactive vitamin B_{12} was given orally with and without normal human gastric juice and also subcutaneously Scintillation from the liver was measured 10 days later The hepatic count after subcutaneous administration was assumed to represent 100 per cent absorption In the 4 subjects the hepatic count per minute per microcurie after oral dosage was 0 to 19 per cent of that seen after the subcutaneous dose When the same oral dose was given with normal human gastric juice the hepatic count was 59 to 63 per cent of that obtained after injection Gastric juice from the patients was given orally with radioactive vitamin B_{12} to other patients with treated pernicious anemia These patients were also given test doses of vitamin B_{12} without gastric juice subcutaneous doses and oral test doses with normal human gastric juice Hepatic counts were low when the radioactive vitamin or the vitamin plus gastric juice from the patients with combined system disease was given Counts after oral ingestion of the vitamin plus normal gastric juice were high When 11 normal subjects were tested hepatic deposition of orally administered radioactive vitamin B_{12} was 40 to 68 per cent of the amount noted after subcutaneous administration

Each of the 4 patients showed neurologic improvement on vitamin B_{12} therapy During follow up periods of from 2 to 96 months neurologic symptoms did not progress

Boston Univer ity School of Medi ine
T fts U i ersity Sch ol of Medicine and
B ston Vete ans Administ ation Hospit l
Boston Mass

60 Foy, H and Kondi, A Nutritional and intestinal factors and iron losses in the genesis of tropical anaemias Lancet 1 423-424 April 14 1956

The etiology and treatment of hypochromic, microcytic iron deficiency anemias occurring in the tropics are discussed In the absence of iron deficiency such anemias might be due to intestinal blood loss caused by parasites defective intestinal iron absorption caused by abnormal calcium phosphorus ratios or loss of iron through feces urine, or sweat, which is estimated to be 1 0 to 1 5 mg per day

Most patients with hypochromic microcytic iron deficiency anemias responded to the daily oral administration of iron or to iron and protein in some form Patients who did not respond to this treatment and who had giant stab cells in the urine often responded to 5 mg of folic acid daily for one week or 80 mcg of vitamin B_{12} or both

61 Foy, H and Kondi A Genesis of tropical anaemias Lancet 2 95 96 July 14 1956 (Letter to Editor)

Microcytic hypochromic iron-deficiency anemia usually responds to iron, but in some cases protein in the form of skimmed milk must also be given A smaller group of these anemias responds fully only if the patient is given, in addition to iron and protein 80 to 100 mcg of intramuscular vitamin B_{12}, in spite of the fact that the vitamin B_{12} serum level is normal Perhaps the reason is that in these anemias the vitamin B_{12} level in the serum must be higher to facilitate protein utilization and metabolism or for the synthesis of methyl and SH groups for hemopoietic purposes

Wellcome Trust Research Laboratories
Nairobi Kenya East Africa

NERVOUS SYSTEM

NEUROPATHIES DUE TO VITAMIN B_{12} DEFICIENCY

62 Bastrup Madsen, P Non anaemic neuropathy caused by deficiency of the anti pernicious anaemia principle following subtotal gastrectomy Acta med Scandinav 147 399 404 Jan 29 1954

Paresthesia occurred intermittently for 9 and 12 months after subtotal gastrectomy in 2 patients One patient also had muscle incoordination and ataxia The neurologic manifestations were similar to those accompanying pernicious anemia but there were no significant hematologic signs In both patients the symptoms disappeared after intramuscular administration of vitamin B_{12} and improvement continued during observation periods of 6 and 12 months

It is concluded that vitamin B_{12} deficiency may cause neuropathy without hematologic changes In this case subtotal gastrectomy may have caused the deficiency

Marselisborg Hospital
Aarhus Denmark

63 Jewesbury E C O Subacute combined degeneration of the cord and achlorhydric peripheral neuropathies without anaemia Lancet 2 307 312 Aug 14, 1954

Subacute combined degeneration of the cord occurred in 5 patients who showed only insignificant changes in the peripheral blood and who had normoblastic marrow All had histamine fast achlorhydria

In one case in which there was no initial treatment with vitamin B_{12} pernicious anemia developed one year later and terminated fatally In another case a small amount of vitamin B_{12} was given

for only two weeks Thereafter the patient remained well for three years but then developed pernicious anemia

In the 3 other patients who received adequate parenteral treatment with vitamin B_{12} pernicious anemia did not develop neurologic signs did not advance and there was symptomatic recovery In 2 of these 3 cases giant metamyelocytes were seen in the marrow this may be one of the earliest signs of pernicious anemia

Two other patients are described in whom peripheral neuropathy was associated with histamine fast achlorhydria and a normal peripheral blood count In 1 of these minimal changes in the sternal marrow also suggested the beginning of pernicious anemia

Patients with histamine fast achlorhydria are liable to develop vitamin B_{12} deficiency but the relative susceptibility of the peripheral nerves spinal cord and marrow is unpredictable Early treatment and close observation of patients with neurologic manifestations is essential A few cases of nonanemic peripheral neuropathy with histamine fast achlorhydria may be due to causes other than vitamin B_{12} deficiency

The author suggests that the terms pernicious anemia and subacute combined degeneration of the cord be dropped in favor of the more comprehensive term vitamin B_{12} deficiency This might be qualified by adding megaloblastic anemia and/or neuropathy (cerebral spinal or peripheral)

Royal Northern Hospital and North Middlesex Hospital London England

Chase Farm Hospital Enfield England

Luton and Dunstable Hospit l Luton England

64 Arias I M Apt L and Pollycove M Absorption of radioactive vitamin B_{12} in nonanemic patients with combined-system disease New England J Med 253 1005 1010 Dec 8 1955

When hematologic manifestations of pernicious anemia do not occur it is difficult to differentiate combined system disease resulting from vitamin B_{12} deficiency from other types of neurologic disorders The authors describe a method for measuring gastrointestinal absorption of radioactive vitamin B_{12} which they used to make this differential diagnosis in 4 patients

Radioactive vitamin B_{12} was given orally with and without normal human gastric juice and also subcutaneously Scintillation from the liver was measured 10 days later The hepatic count after subcutaneous administration was assumed to represent 100 per cent absorption In the 4 subjects the hepatic count per minute per microcurie after oral dosage was 0 to 19 per cent of that seen after the subcutaneous dose When the same oral dose was given with normal human gastric juice the hepatic count was 59 to 63 per cent of that obtained after injection Gastric juice from the patients was given orally with radioactive vitamin B_{12} to other patients with treated pernicious anemia These patients were also given test doses of vitamin B_{12} without gastric juice subcutaneous doses and oral test doses with normal human gastric juice Hepatic counts were low when the radioactive vitamin or the vitamin plus gastric juice from the patients with combined system disease was given Counts after oral ingestion of the vitamin plus normal gastric juice were high When 11 normal subjects were tested hepatic deposition of orally administered radioactive vitamin B_{12} was 40 to 68 per cent of the amount noted after subcutaneous administration

Each of the 4 patients showed neurologic improvement on vitamin B_{12} therapy During follow up periods of from 2 to 96 months neurologic symptoms did not progress

Boston University School of Medi ine Tufts U i ersity School of Medicine and B st n Vete ans Admi i tration Hospital Bosto Mass

65 Holmes, J M Cerebral manifestations of vitamin B_{12} deficiency, Brit M J 2 1394-1398 Dec 15, 1956

Brain lesions are a less familiar part of the neurologic syndrome of vitamin B_{12} deficiency than lesions of the spinal cord and peripheral nerves (usually termed subacute combined degeneration') However, untreated brain lesions may result in severe dementia Evidence indicates that the mental changes are specifically related to a disturbance of cerebral metabolism which is corrected by adequate treatment with vitamin B_{12}

Of 25 patients with vitamin B_{12} deficiency syndrome involving the nervous system 14 showed cerebral symptoms including slow mental processes confusion and memory defect, depression delusions hallucinations opticatrophy epilepsy dysphasia agitation and maniacal behavior Cerebral symptoms sometimes preceded addisonian anemia or spinal and peripheral nerve involvement by several years Diagnosis was established by signs of spinal involvement or peripheral neuropathy, evidence of addisonian anemia in blood count or bone marrow and histamine fast achlorhydria Doubtful cases require application of additional diagnostic methods The author describes 2 patients who showed cerebral symptoms with normal blood and bone marrow findings Treatment with 100 and 500 mcg of vitamin B_{12} resulted in rapid improvement

Spinal or cerebral manifestations require treatment with much higher doses of vitamin B_{12} than those needed for maintenance of a normal blood count Vitamin B_{12} should be given in an initial dose of 1 000 mcg followed by 500 mcg twice weekly for the first month and thereafter by not less than 100 mcg weekly for the next six months If monthly injections are given 1 000 mcg doses are advisable Oral preparations of intrinsic factor with vitamin B_{12} are still not fully evaluated and should not be used in the presence of neurologic symptoms

Recent literature presents evidence that these cerebral symptoms are specifically related to vitamin B_{12} deficiency and not to anemia

Birmingham Regional Hospital
Birmingham England
Staffordshire General Infirmary
Staffordshire England

NEUROPATHIES GENERAL

66 Lereboullet, J and Pluvinage, R Use of massive doses of vitamin B_{12} in neurology (1 000 micrograms per injection), Semaine d hôp Paris 29 1849, June 6 1953 (abstr J A M A 153 601, Oct 10, 1953)

Increasing supplies of vitamin B_{12} have made possible its use in larger doses and permitted extension of its sphere of action The blood picture in pernicious anemia was improved more rapidly and effectively by large doses of this vitamin and some nervous lesions previously considered irreversible showed regression Equally significant results have been obtained in other neurologic disturbances multiple sclerosis the polyneuritic and psychic complications of alcoholism syndromes in the subacute superior polio-encephalitic group and essential facial neuralgia Vitamin B_{12} is apparently entirely nontoxic and complications resulting from its use even in doses of 1 000 mcg have not been reported

67 Benbanasté J V La vitamina B_{12} en clinica neurologica [Vitamin B_{12} in clinical neurology] Prensa méd argent 41 1666-1668, June 11, 1954

A patient with tabes obtained complete relief of paresthesia while receiving 10 mcg of vitamin B_{12} twice daily but other symptoms were unaffected Six of 7 other patients with tabes obtained rapid pain relief on 600 mcg daily and also showed objective improvement A patient with neuroanemia receiving 30 mcg of vitamin B_{12} daily had progressive relief of anemia and less consistent relief of

paresthesia abnormal tendon reflexes and walking difficulties Five patients with facial neuralgia who received 300 to 600 mcg of vitamin B_{12} daily were considered cured after 15 days A child with intention tremor dating from an attack of typhoid fever was still free of tremor four months after a week s course of 1 000 mcg of vitamin B_{12} daily Two patients with brachiocephalic neuralgia who had obtained no relief from analgesics alone improved greatly when given 400 mcg of vitamin B_{12} in addition to the analgesics

Hospital Regional Español
Bahia Blanca, Argentina

68 Schmidt, W and Winter H Vorläufige Erfahrungen mit einem hochdosierten Vitamin B_{12} Präparat (Docigram 1000) [Preliminary results with a high dosage vitamin B_{12} preparation] Ther d Gegenw 93 265 1954 (abstr Schweiz. med Wchnschr 85 137 Feb 5 1955)

Vitamin B_{12} 1 000 mcg was given to 3 patients with pernicious anemia to 3 with herpes zoster and to 3 with neuralgia of the brachial plexus It was effective in all these patients It was also effective in 2 out of 4 patients with trigeminal neuralgia and in 1 of 2 with polyneuritis

69 *Wilson S J Grady H. J Rose* D L. and Heath, H. E A metabolic and therapeutic study of various cobalamins in multiple sclerosis J Lab & Clin. Med 44 954 Dec 1954 (in Soc. Proc)

'The blood of patients with multiple sclerosis has been said to contain toxic levels of cyanide the amount being about one tenth fatal levels Mushett and associates studied the antidotal effects of vitamin B_{12a} (hydroxocobalamin) in experimental cyanide poisoning and were able to both revive and protect their animals *These two observations logically caused* us to study the metabolism and therapeutic value of cobalamins other than cyanocobalamin in patients with multiple sclerosis All cobalamins have a marked affinity for CN being converted instantaneously to the ordinary vitamin B_{12} (cyanocobalamin) The molecular weights are such that 1 000 mcg of cobalamins can bind only 18 mcg of HCN

In the present study a mixture of sulfitocobalamin and hydroxocobalamin was given intramuscularly to 13 patients with multiple sclerosis The daily dosage was 2 000 mcg In 3 subjects the urinary excretion of CN^- cyanocobalamin and cyanide-free cobalamins was determined The method of Boxer and Richards was used in determining these substances and also in preparing cyanide free cobalamins Base line studies revealed the following average 24 hour total urinary excretion CN^-, 2 0 mcg cyanocobalamin 30 5 mcg and cyanide free cobalamin 131 mcg During the injection of 2 000 mcg of cyanide free cobalamins per day the following results were obtained CN 3 6 mcg cyanocobalamin 231 mcg and cyanide free cobalamins 714 mcg

'The patients were treated from 2 to 8 months One patient received treatment for 16 months Inasmuch as exacerbations of the disease occurred during this period of treatment it was concluded that cyanide free cobalamins have no beneficial effect and remissions observed were spontaneous One patient was sensitive to B_{12} and an explosive neurological involvement occurred It was further concluded that no significant amounts of cyanide are present to convert appreciable amounts of cyanide free cobalamins to cyanocobalamin

Kansas City Kan

70 Levin, M B Vitamin B_{12} in neurometabolism (preliminary clinical report) Am J Digest Dis 22 96-97, April 1955

The author reviews his experience with intramuscular injections of vitamin B_{12} in the treatment of patients with neurometabolic disorders Dosages between 2 000 and 10 000 mcg were given two to three times weekly Doses of 3 000 to 5 000

mcg were usually effective Vitamin B_{12} administered orally failed to give comparable results

Three patients with pernicious anemia and spinal cord involvement benefited from vitamin B_{12} and crude liver extract In 3 patients with multiple sclerosis striking improvement in speech, swallowing salivation walking and nocturnal epileptiform seizures was obtained A remission of two weeks was maintained in one patient Improvement was also noted in patients with cerebral edema and hemorrhage with hemiplegia These patients received the vitamin B_{12} injections before and after the use of cortisone to absorb the clot An infant with amyotonia improved noticeably under this regimen After three injections a woman paralyzed by amyotrophic lateral sclerosis showed improvement One patient with encephalomyelitis was given antibiotic therapy combined with vitamin B_{12} and cortisone Benefit could not be attributed to any single agent This combination of therapy is suggested for patients with poliomyelitis Most of the patients with radiculitis showed marked improvement within 7 to 10 days Results were more favorable when thiamine and vitamin B_{12} were given simultaneously

Vitamin B_{12} therapy was successful in treatment of retrobulbar optic neuritis vestibular dizziness trigeminal neuralgia Bell s palsy and peripheral neuritis Paroxysmal tachycardia with repeated irritation of the cardiac sympathetic nerves was reduced in 2 patients Attacks resembling insulin shock occurred in several patients when the injections were given before breakfast

Baltimore Md

71 Murphy, G Polineuropatia alcóholica su tratamiento con vitamina B_{12} [Alcoholic polyneuritis treatment with vitamin B_{12}] Prensa méd argent 41 804-806 March 19, 1954

On the theory that alcoholic polyneuritis is a nutritional deficiency 9 patients were treated with 50 or 100 mcg of vitamin B_{12} daily for 12 to 36 days These 9 were chosen because of the seriousness of their symptoms and because none had received vitamins or liver extracts recently All were men who had drunk considerable amounts daily for 6 to 30 years and often became drunk Four were admitted with delirium tremens 4 because of polyneuritis and 1 in a ' confused state Of the 9 patients, 7 obtained maximum subjective and objective relief Pains ceased in one patient within 12 hours after the first 100 mcg dose of vitamin B_{12} One patient was apparently unaffected by therapy even when B complex was added and one received only partial relief The author emphasizes that these two had been alcoholics for 20 and 30 years and each had portal cirrhosis He points out that although a similar patient obtained relief it is generally true the shorter the period of alcoholism the better the results Symptoms in the arms were alleviated more quickly and more completely than were symptoms in the legs

The authors conclude that vitamin B_{12} is a therapeutic agent highly efficient in the treatment of alcoholic polyneuritis

72 Bacher, K R Vitamin B_{12} for treatment of alcoholic delirium Deutsche med Wchnschr 79 1901 1903 Dec 17, 1954 (abstr J A M A 157 969 March 12, 1955)

Delirium tremens following injury or surgical trauma in 6 patients was treated with vitamin B_{12} in intramuscular injections of 1 000 mcg One to four injections were required in 3 patients the number required by the others is not stated

Delirium subsided in 5 patients 1 (cachectic and gastrectomized for cancer) died Dehydrocholic acid given intravenously to 3 of the patients before the trial of vitamin B_{12} was ineffective

It is recommended that vitamin B_{12} be given prophylactically to patients running the risk of delirium tremens who

must undergo operation or receive treat ment for an injury

St ttgart Germany

DIABETIC NEUROPATHIES

73 Coste P D and Klinger R Use of massive doses (500 to 1 000 mcg) of vitamin B_{12} for various conditions present in diabetic patients Policlinico (sez. prat) 61 143 149 Feb 1, 1954 (abstr J A M A 155 781 June 19 1954)

Fifteen patients with diabetes of long standing were given from 500 to 1 000 mcg of vitamin B_{12} daily to treat various other conditions present Five patients had lumbar and sciatic pain due to lum bosacral arthrosis 2 had neuralgia of the fifth nerve 2 had pruritus vulvae and 1 each had polyneuritis with vertebral poly arthrosis secondary brachial neuritis sec ondary intercostal neuritis herpes zoster diffuse psoriasis and parkinsonian trem or Diet and insulin dosage were not altered The vitamin caused no toxic effects Good results were obtained in pa tients with neurologic conditions In these pain was succeeded by paresthesia which disappeared as therapy was continued This was ascribed to the direct action of the vitamin on the nervous tissues Grati fying results were also observed in the 1 patient with herpes zoster and in the 2 with pruritus vulvae The vitamin had no effect however on diffuse psoriasis and parkin sonian tremor Changes in the carbo hydrate metabolism attributable to the vitamin were not observed

74 Shuman C R and Gilpin S F Di abetic neuropathy controlled thera peutic trials Am J M Sc 227 612 617 June 1954

Thirty seven patients with diabetes mellitus had neuropathy which did not respond to adequate treatment of the diabetes Vitamin B_{12} was administered orally or intramuscularly to 12 of these patients in daily doses ranging from 10 to 1 000 mcg but no improvement occurred in their neurologic disorders Pregnant mammalian liver adenosine tri phosphate with thiamine and pantothenic acid were also ineffective in producing objective improvement

Temple University Hospital and School of Medicine Philadelphia Pa

75 Sauer H and Dussler, A Disease aspect of diabetic polyneuritis and its treatment with vitamin B_{12} Deutsche med Wchnschr 79 1046-1048 June 25 1954 (abstr J A M A 156 643, Oct 9 1954)

Diabetic polyneuritis in 18 patients was treated with vitamin B_{12} given intra muscularly or subcutaneously The dos age was usually 30 to 60 mcg daily for two weeks a few patients received 1 000 mcg daily Fifteen patients improved and 3 (2 of them despite high dosage) did not

St ttgart Germany

76 Miller D S and Lichtman W F Diabetic neuropathic arthropathy of feet Summary report of seventeen cases A M A Arch Surg 70 513 518 April 1955

The increased incidence of foot defor mities accompanying diabetes is pointed out Such arthropathies usually occur in poorly controlled long standing diabetes and are due to involvement of the auto-nomic nervous system rather than to poor circulation Out of 17 of the authors pa tients with this complication 16 had re fused or neglected treatment of the dia betes The usual symptoms and signs were reduced vibratory and position sense di minished reflexes and a widened short ened everted foot often accompanied by bone displacement or destruction Rec ommended treatment includes better con trol of the diabetes good foot hygiene and if necessary amputation or sympa thectomy Supportive treatment consists of administration of vitamins B_1 and B_{12}

dilute hydrochloric acid, crude liver extract and injections of pregnant mammalian liver extract Vasodilators and antibiotics may also be indicated

Cook County Hospital and
Chicago Medical School
Chicago Ill

77 Lueck, A G Neurologic disturbances complicating diabetes J Iowa M Soc 46 296-300, June 1956

In discussing the neurologic disturbances complicating diabetes the author divides the disturbances into two groups peripheral neuritis and autonomic neuropathy and presents 18 cases The diabetes was brought under control and in 16 patients injections of vitamin B_{12} were added to the regimen In most cases pain either subsided or disappeared In one case strabismus disappeared after two months of therapy

The author states that this therapy is of questionable value but it is my impression that for the present it should be used Practically all diabetics with neuropathy are taking insulin or should be and they can easily inject vitamin B_{12} once a day for themselves without great cost Beyond these measures nothing on the horizon holds any promise of significance

Des Moines Iowa

TRIGEMINAL NEURALGIA

78 Alexander E, Jr and Davis, C H, Jr Trigeminal neuralgia conservative management with massive vitamin B_{12} therapy North Carolina M J 14 206-207, May 1953 (abstr Clin Med 61 400 May 1954)

The apparent effectiveness of small doses of vitamin B_{12} in relieving the neuropathy of pernicious anemia and in the treatment of certain peripheral neuritides suggested its use in trigeminal neuralgia Accordingly 17 patients with trigeminal neuralgia have been treated with 100 mcg of vitamin B_{12} given intramuscularly over a 10-day period Of these 6 had complete relief lasting from 2 to 8 months and 2 had satisfactory relief In 4 patients previously treated with surgery or alcohol nerve blocks pain relief was delayed but was eventually satisfactory The results warrant continued investigation of this treatment for trigeminal neuralgia.

79 Surtees S J and Hughes R R. Treatment of trigeminal neuralgia with vitamin B_{12}, Lancet 1 439-441, Feb 27 1954

Vitamin B_{12} administered to 18 patients with trigeminal neuralgia was followed by considerable improvement or complete relief of pain in 14, little or no immediate improvement in 3 and moderate improvement in 1 A patient with glossopharyngeal neuralgia also received marked relief after vitamin B_{12} therapy Some patients received 1 000 mcg of vitamin B_{12} intramuscularly twice weekly for five doses, some 1 000 mcg daily for 10 days followed by 1 000 mcg twice weekly for five doses and a few 1 000 mcg twice daily for periods up to three weeks

Injections of 1 000 mcg of vitamin B_{12} administered twice weekly for five doses failed to relieve the pain of 3 patients with postherpetic neuralgia and of 2 with facial neuralgia precipitated by operative procedures The authors emphasize that failure may have been caused by inadequate dosage

Royal Southern Hospital
Liverpool England

80 Albrecht K. and Krump, J Diagnose Differentialdiagnose und Behandlungsmöglichkeiten der Trigeminusneuralgie (Unter besonderer Berucksichtigung der konservativen Behandlung mit Hydantoinpräparaten und Vitamin B_{12}) [Diagnosis, differential diagnosis and therapeutic possibilities in trigeminus neuralgia conservative treatment with hydan

toin drugs and vitamin B_{12}] München med Wchnschr 96 1037 1039, Sept 3 1954

Vitamin B_{12} was given in daily intra muscular doses of 1 000 mcg for 10 days to 13 patients with trigeminal neuralgia Improvement began after about the fourth injection Of the 13 patients 7 (including 2 whose neuralgia followed herpes zoster) were completely relieved 1 with long standing neuralgia was so much im proved that she refused surgery 1 pa tient was not benefited No comment is made on the results in the remaining patients although early in the article the authors state that vitamin B_{12} was used with good effect in trigeminal neuralgia

Erlangen, Germany

HERPES ZOSTER

81 Schiller F Herpes zoster Review with preliminary report on new meth od for treatment of postherpetic neu ralgia J Am Geriatrics Soc 2 726-735 Nov 1954

Pathogenesis and diagnosis of herpes zoster are discussed with emphasis on the treatment of postherpetic neuralgia Among the many treatments tried vita min B_{12} is reported to have some merit The author has observed 1 patient with relatively mild postherpetic pain who de rived some benefit Its use as an anti neuralgic agent is recommended on the basis that it may improve the metabolism of nerve cells which are not irreversibly damaged It is also noted that in large doses (1 000 mcg once or twice weekly) vitamin B_{12} induces a certain well being in the elderly

The author s proposed method of treatment of neuralgic pain which has been used with some benefit in 10 patients is segmental counterirritation injection of small amounts of hypertonic saline into paravertebral muscle

Uni e sity of California Medical School San Francisco Calif

82 Jolles K E Herpes zoster treated with vitamin B_{12} Brit M J 1 166 167, Jan 15 1955 (In Correspondence)

The writer comments further on his successful treatment of herpes zoster with three or four intramuscular doses of 50 mcg of vitamin B_{12} Doubling the dose did not produce more beneficial effects Oral treatment (150 mcg four times daily) was also effective within eight days in one elderly woman with herpes zoster oph thalmicus

W est B om ich England

83 Treatment of herpes zoster J A M A 163 482 Feb 9 1957 (in Foreign Letters India)

J R Srivastava gave vitamin B_{12} to 10 patients with herpes zoster (*J Indian M A* 27 10 1956) There was marked relief from pain rapid disappearance of vesicles and crusts and no postherpetic pain The results on the whole were excellent

84 Wigoder R G B and Jeffs H G Treatment of herpes zoster Brit M J 1 523 March 2 1957 (in Corre-spondence)

The authors treated 21 patients with herpes zoster with daily 100 mcg injec tions of vitamin B_{12} for six days Erup tions were painted with flexible collodion twice daily

Pain ceased towards the end of the treatment period in all but 2 patients in whom it was especially severe and per sisted for three months In these cases one 1 000 mcg injection was given on the first and third days and three 250 mcg injections on three alternate days

GASTRECTOMY

85 Ley, A B and Sharpe L E The absorption of vitamin B_{12} after total gastrectomy, Clin Research Proc 2 31, March 1954

In a study of the absorption of vitamin B_{12} after total gastrectomy, Co^{60} vitamin B_{12} 0 5 to 1 0 mcg, was given to 6 totally gastrectomized patients to 1 patient with pernicious anemia, and to 2 normal persons Stools were subsequently examined for radioactivity Normal subjects absorbed approximately 65 per cent of the dose The patient with pernicious anemia, in early remission, absorbed approximately 22 per cent, when the same dose was administered with intrinsic factor the absorption increased to 49 per cent Gastrectomized patients absorbed from 0 to 22 per cent with intrinsic factor their absorption varied from 36 to 59 per cent

One patient gastrectomized 5 years before this study had a mild macrocytic anemia with a hematocrit of 35 per cent The bone marrow aspirate on this patient showed equivocal megaloblastosis The patient was treated with vitamin B_{12} 5 mcg by mouth each day During the ensuing seven weeks the hematocrit fell to 32 8 per cent Treatment for the next eight weeks with the same daily dose of vitamin B_{12} in addition to an intrinsic factor concentrate was followed by a rise in the hematocrit to 43 per cent with the mean corpuscular volume decreasing to nearly normal The authors believe these findings demonstrate the importance of the gastric mucosa in the absorption of vitamin B_{12}

Memorial Center for Cancer and Allied Diseases
New York N Y

86 McCorkle, H J and Harper H A The problem of nutrition followi complete gastrectomy, Ann. Su 140 467-474 Oct 1954

A review of the nutritional status 24 totally gastrectomized patients in cated that only 6 had gained signific amounts of weight postoperatively O 9 could eat comfortably

To improve digestion and nutriti following total gastrectomy a study v made of 120 dogs subjected to the ope tion The study showed that alimentation time was decreased although absorption of amino acids was more rapid in gastrectomized animals than in controls Increased nitrogen loss in the stool showed that it was less efficient Most of the dogs remained in positive nitrogen balance however, provided they ate enough protein Rates of absorption of fat and of glucose were increased Severe postoperative diarrhea was prevented by giving 3 Gm of neomycin daily for two days once each month and a few drops of hydrochloric acid daily Anemia occurred after gastrectomy but could be minimized by vitamin therapy The dogs were given intrinsic factor and 10 mcg of vitamin B_{12} every third day but the authors believe that larger oral doses of vitamin B_{12} might have prevented anemia even without intrinsic factor

The results indicate that good nutrition and weight can be maintained after total gastrectomy if isoperistaltic continuity between the esophagus and duodenum is achieved and if the animals can be trained to eat slowly and often

Three patients attained good nutritional status after gastrectomy and anastomosis of an isoperistaltic segment of colon between the esophagus and duodenu had adequate food capacity

and appetite and digestive comfort The importance of giving large oral doses of vitamin B_{12} to patients who had undergone total gastrectomy is emphasized

University of California School of Medicine San Francisco Calif

87 Paulson M and Harvey J C **Hematological alterations after total gastrectomy Evolutionary sequences over a decade** J A M A 156 1556-1560 Dec 25 1954

Hematologic study of 27 totally gastrectomized (for carcinoma of the stomach) patients revealed that an iron deficiency anemia developed shortly after operation then within two years macrocytosis developed in the peripheral blood and if survival was long enough a true macrocytic megaloblastic anemia developed The iron deficiency could be controlled by routine administration of ferrous gluconate or ferrous sulfate The macrocytic megaloblastic anemia was responsive to parenteral vitamin B_{12} Treatment given to 5 patients is shown in a table Initially 4 of these patients were given weekly intramuscular injections of vitamin B_{12} ranging from 30 to 150 mcg for about seven months followed by the same dose once a month for 6 6 26 and 24 months respectively Two of these patients also received oral folic acid 15 mg daily Oral vitamin B_{12} was also tried (unsuccessfully) in one of the 4 patients The fifth patient was treated unsuccessfully with folic acid but later he responded to crude liver

A study of intestinal absorption of vitamin B_{12} was made in one gastrectomized patient Co^{60} vitamin B_{12} 0 5 mcg was given orally About 87 per cent of the radioactive vitamin was recovered in the stool (normal recovery 10 to 40 per cent)

Johns Hopkins University and Hospital Baltimore Md

88 Tomoda M **Agastric pernicious anemia** Rept of Hematological Symposium 6 384-410 1954 (abstr Blood 11 96 Jan 1956)

Pernicious anemia occurred in all of 4 patients observed for more than 5 years after total gastrectomy Development of anemia after operation was as follows hypochromic or orthochromic anemia in the 1st or 2nd year hyperchromic anemia in the 3rd pernicious anemia in the 5th or 6th Folic acid concentration in the blood dropped during the early stage more than 3 years after operation not only folic acid but also vitamin B_{12} was decreased In the reported 4 cases anemia was treated successfully by folic acid or vitamin B_{12} Relapse occurred after interruption of treatment

Kyushu University School of Medicine Fukuoka Japan

89 Mauri Paolini A and Marinoni E **Hematologic changes following total gastrectomy** Chirurgia 10 331 1955 (Internat Abstr Surg 103 359 1956 in Surg Gynec & Obst Oct 1956)

After a careful review of many reports of hyperchromic megaloblastic anemia following total gastrectomy the authors conclude that development of this anemia is directly related to the operation Most cases occur 5 or 6 years after gastrectomy and patients respond well to treatment with vitamin B_{12} and liver extract The authors recommend parenteral administration

90 Pitney W R and Beard M F ***Vitamin B_{12} deficiency following total gastrectomy*** A M A Arch Int Med 95 591 593 April 1955

Lack of intrinsic factor causes decreased absorption of vitamin B_{12} after total gastrectomy but megaloblastic anemia usually does not result until at least two years after operation because the body possesses a considerable reserve of vitamin B_{12} As this is gradually depleted serum levels of vitamin B_{12} fall thus measurement of these levels after opera

GASTRECTOMY

85 Ley, A B and Sharpe L E The absorption of vitamin B_{12} after total gastrectomy, Clin Research Proc 2 31, March 1954

In a study of the absorption of vitamin B_{12} after total gastrectomy, Co^{60} vitamin B_{12} 0 5 to 1 0 mcg was given to 6 totally gastrectomized patients, to 1 patient with pernicious anemia and to 2 normal persons Stools were subsequently examined for radioactivity Normal subjects absorbed approximately 65 per cent of the dose The patient with pernicious anemia in early remission absorbed approximately 22 per cent, when the same dose was administered with intrinsic factor the absorption increased to 49 per cent Gastrectomized patients absorbed from 0 to 22 per cent with intrinsic factor their absorption varied from 36 to 59 per cent

One patient gastrectomized 5 years before this study, had a mild macrocytic anemia with a hematocrit of 35 per cent The bone marrow aspirate on this patient showed equivocal megaloblastosis The patient was treated with vitamin B_{12} 5 mcg by mouth each day During the ensuing seven weeks the hematocrit fell to 32 8 per cent Treatment for the next eight weeks with the same daily dose of vitamin B_{12} in addition to an intrinsic factor concentrate was followed by a rise in the hematocrit to 43 per cent with the mean corpuscular volume decreasing to nearly normal The authors believe these findings demonstrate the importance of the gastric mucosa in the absorption of vitamin B_{12}

Memorial Center for Cancer and Allied Diseases New York N Y

86 McCorkle, H J and Harper, H A The problem of nutrition following complete gastrectomy Ann Surg 140 467-474, Oct 1954

A review of the nutritional status of 24 totally gastrectomized patients indicated that only 6 had gained significant amounts of weight postoperatively Only 9 could eat comfortably

To improve digestion and nutrition following total gastrectomy a study was made of 120 dogs subjected to the operation The study showed that alimentation time was decreased although absorption of amino acids was more rapid in gastrectomized animals than in controls Increased nitrogen loss in the stool showed that it was less efficient Most of the dogs remained in positive nitrogen balance however provided they ate enough protein Rates of absorption of fat and of glucose were increased Severe postoperative diarrhea was prevented by giving 3 Gm of neomycin daily for two days once each month and a few drops of hydrochloric acid daily Anemia occurred after gastrectomy but could be minimized by vitamin therapy The dogs were given intrinsic factor and 10 mcg of vitamin B_{12} every third day but the authors believe that larger oral doses of vitamin B_{12} might have prevented anemia even without intrinsic factor

The results indicate that good nutrition and weight can be maintained after total gastrectomy if isoperistaltic continuity between the esophagus and duodenum is achieved and if the animals can be trained to eat slowly and often

Three patients attained good nutritional status after gastrectomy and anastomosis of an isoperistaltic segment of colon between the esophagus and duodenum They had adequate food capacity

and appetite and digestive comfort The importance of giving large oral doses of vitamin B_{12} to patients who had undergone total gastrectomy is emphasized

University of California School of Medicine San Francisco Calif

87 Paulson M and Harvey, J C Hematological alterations after total gastrectomy Evolutionary sequences over a decade J A M A 156 1556-1560 Dec 25 1954

Hematologic study of 27 totally gastrectomized (for carcinoma of the stomach) patients revealed that an iron deficiency anemia developed shortly after operation then within two years macrocytosis developed in the peripheral blood and if survival was long enough a true macrocytic megaloblastic anemia developed The iron deficiency could be controlled by routine administration of ferrous gluconate or ferrous sulfate The macrocytic megaloblastic anemia was responsive to parenteral vitamin B_{12} Treatment given to 5 patients is shown in a table Initially 4 of these patients were given weekly intramuscular injections of vitamin B_{12} ranging from 30 to 150 mcg for about seven months followed by the same dose once a month for 6 6 26 and 24 months respectively Two of these patients also received oral folic acid 15 mg daily Oral vitamin B_{12} was also tried (unsuccessfully) in one of the 4 patients The fifth patient was treated unsuccessfully with folic acid but later he responded to crude liver

A study of intestinal absorption of vitamin B_{12} was made in one gastrectomized patient Co^{60} vitamin B_{12} 0 5 mcg was given orally About 87 per cent of the radioactive vitamin was recovered in the stool (normal recovery 10 to 40 per cent)

Johns Hopkins University and Hospital Baltimore Md

88 Tomoda M Agastric pernicious anemia Rept of Hematological Symposium 6 384-410 1954 (abstr Blood 11 96 Jan 1956)

Pernicious anemia occurred in all of 4 patients observed for more than 5 years after total gastrectomy Development of anemia after operation was as follows hypochromic or orthochromic anemia in the 1st or 2nd year hyperchromic anemia in the 3rd pernicious anemia in the 5th or 6th Folic acid concentration in the blood dropped during the early stage more than 3 years after operation not only folic acid but also vitamin B_{12} was decreased In the reported 4 cases anemia was treated successfully by folic acid or vitamin B_{12} Relapse occurred after interruption of treatment

Kyushu University School of Medicine Fukuoka Japan

89 Mauri Paolini A and Marinoni E *Hematologic changes following total* gastrectomy Chirurgia 10 331, 1955 (Internat Abstr Surg 103 359 1956 in Surg Gynec & Obst Oct 1956)

After a careful review of many reports of hyperchromic megaloblastic anemia following total gastrectomy the authors conclude that development of this anemia is directly related to the operation Most cases occur 5 or 6 years after gastrectomy and patients respond well to treatment with vitamin B_{12} and liver extract The authors recommend parenteral administration

90 Pitney W R and Beard M F Vitamin B_{12} *deficiency following total* gastrectomy A M A Arch Int Med 95 591 593 April 1955

Lack of intrinsic factor causes decreased absorption of vitamin B_{12} after total gastrectomy but megaloblastic anemia usually does not result until at least two years after operation because the body possesses a considerable reserve of vitamin B_{12} As this is gradually depleted serum levels of vitamin B_{12} fall thus measurement of these levels after opera

tion provides an accurate index of the state of vitamin B_{12} nutrition

One 57 year old woman gastrectomized for carcinoma did not develop megaloblastic anemia but her serum concentrations of vitamin B_{12} progressively fell from over 150 micromicrograms per cc 4 months after operation to less than 50 micromicrograms about 18 months after operation (when she died of metastases) The Schilling test showed that she absorbed normal amounts of orally administered vitamin B_{12} only when it was given with normal gastric juice

In view of these findings the authors recommend administration of vitamin B_{12} to gastrectomized patients even when blood cell count is normal This will not only prevent development of megaloblastic anemia but will also prevent other manifestations of vitamin B_{12} deficiency such as malaise anorexia and lassitude which might be mistaken for signs of metastasized carcinoma in some cases

91 Badenoch, J Evans J R Richards W C D and Witts L J Megaloblastic anemia following partial gastrectomy and gastro enterostomy, Brit J Haemat 1 339, 344 Oct 1955 (abstr J A M A 160 321-322, Jan 28 1956)

Although megaloblastic anemia due to deficiency in vitamin B_{12} is almost inevitable after total gastrectomy if the patient survives the operation for six years or longer, it is rare after operations in which a portion of the stomach remains Recently the authors observed five patients in whom megaloblastic anemia developed after partial gastrectomy and one patient in whom it developed after gastroenterostomy Since intrinsic factor is secreted by the body of the stomach it seemed unlikely that the megaloblastic anemia following partial gastrectomy could be due to lack of this factor However new techniques of investigation made it possible to study such anemias more fully All the patients had a macrocytic anemia with megaloblasts in the sternal marrow They were treated with vitamin B_{12} and four showed optimal response A fifth showed clinical improvement but although the bone marrow changed from megaloblastic to normoblastic erythropoiesis there was no significant alteration in the peripheral blood a course of folic acid was ineffective but treatment of the accompanying iron deficiency resulted in a good response The sixth patient whose anemia was mild showed no therapeutic response to either vitamin B_{12} or folic acid The interval between operation and the diagnosis of megaloblastic anemia ranged from 2 to 17 years In all instances the total duration of dyspepsia before and after operation had been 20 years or longer The authors investigated the excretion of acid and uropepsin, and the absorption and excretion of fat They also attempted to obtain biopsy specimens from the remaining portion of the stomach and estimated the vitamin B_{12} content of the serum and the secretion of intrinsic factor The results of the studies indicated that in five of the subjects the anemia was caused by vitamin B_{12} deficiency There was failure to absorb vitamin B_{12} which was corrected by the addition of intrinsic factor the biopsy studies revealed atrophy of the gastric body mucosa comparable in severity to that seen in pernicious anemia In the sixth patient the nature of the deficiency and its pathogenesis remained obscure Steatorrhea was not a significant factor in any of the cases Since megaloblastic anemia is rare after partial gastrectomy and gastroenterostomy it seemed possible that the patients might have Addisonian pernicious anemia and that the gastric operation was only incidental This hypothesis was not tenable in view of the absence of a family history of pernicious anemia and because the histological study of the stomach removed at partial gastrectomy showed normal gastric body glands and in no way resembled the mucosal atrophy seen in pernicious anemia The biopsy

studies did not allow a detailed comparison between the stomach in this condition and in idiopathic pernicious anemia but the authors gained the impression that gastritis was present and they feel that chronic gastritis associated with prolonged dyspepsia gastric ulceration and operative intervention may have contributed to the development of gastric atrophy

92 MacLean L. D The differentiation of achylia gastrica and achlorhydria by means of radioactive vitamin B_{12} Gastroenterology 29 653 665 Oct 1955

The vitamin B_{12} excretion test differentiates patients with simple achlorhydria and achylia gastrica The latter are possibly more likely to develop gastric cancer Pernicious anemia patients develop gastric cancer more commonly than those who do not have pernicious anemia

This study showed that gastrectomized patients lack the intrinsic factor necessary for absorption of oral vitamin B_{12} A follow up study of patients who survived for over three years after total gastrectomy without vitamin B_{12} or liver extract therapy has shown that 90 per cent develop megaloblastic marrow Apparently pernicious anemia is a late but an inevitable sequela of total or proximal subtotal gastrectomy but is not likely to occur until at least 3 years after the operation

University of Minnesota
Minneapolis Minn

93 MacLean L. D and Sundberg R D Incidence of megaloblastic anemia after total gastrectomy New England J Med 254 885-893 May 10 1956

The authors review of 17 cases of megaloblastic anemia following total gastrectomy reported in the literature suggests that if the patient survives long enough megaloblastic anemia will occur

The authors state that 10 of 11 totally gastrectomized patients (observed by them) who did not receive prophylactic therapy for pernicious anemia and who survived for over three years developed a megaloblastic bone marrow Megaloblastosis occurred irrespective of the type of enteric reconstruction and was not due to blind loop anemia

Six patients with total gastrectomy and 3 with proximal subtotal gastrectomy were unable to absorb oral Co^{60} vitamin B_{12} until intrinsic factor was added This was evidence that the site of intrinsic factor production is the body and fundus the area of pathologic change in the stomach in pernicious anemia The human antrum apparently is not important in elaboration of intrinsic factor

Vitamin B_{12} or purified liver extract alone produced remissions in these patients The authors state that all patients subjected to total gastrectomy or proximal subtotal gastrectomy with esophago-antrostomy should receive continuous prophylactic parenteral vitamin B_{12} or liver extract therapy commencing within three years after operation

University of Minnesota Medical School
Minneapolis Minn

94 Harvey J C The vitamin B_{12} deficiency state engendered by total gastrectomy Surgery 40 977 989 Nov 1956 (in Recent Advances in Surgery)

In 30 patients treatment with liver extracts or vitamin B_{12} was purposely withheld after total gastrectomy in order to observe the development of vitamin B_{12} deficiency Of the 30 patients 10 were alive at the time of writing their postoperative survival time ranging between 2½ years and 17 years

The first evidence of vitamin B_{12} deficiency was macrocytosis of erythrocytes which occurred from six months to seven years (average two and one half years) after gastrectomy Anemia appeared next within an average of four years postoperatively and megaloblasts invariably were found in the marrow within one year after that Three patients who failed to come in for observation according to in

tion provides an accurate index of the state of vitamin B_{12} nutrition

One 57 year-old woman gastrectomized for carcinoma did not develop megaloblastic anemia but her serum concentrations of vitamin B_{12} progressively fell from over 150 micromicrograms per cc 4 months after operation to less than 50 micromicrograms about 18 months after operation (when she died of metastases) The Schilling test showed that she absorbed normal amounts of orally administered vitamin B_{12} only when it was given with normal gastric juice

In view of these findings the authors recommend administration of vitamin B_{12} to gastrectomized patients even when blood cell count is normal This will not only prevent development of megaloblastic anemia but will also prevent other manifestations of vitamin B_{12} deficiency such as malaise anorexia and lassitude which might be mistaken for signs of metastasized carcinoma in some cases

91 Badenoch J Evans J R, Richards, W C D and Witts L J Megaloblastic anemia following partial gastrectomy and gastro enterostomy, Brit J Haemat 1 339 344, Oct 1955 (abstr J A M A 160 321 322, Jan 28, 1956)

Although megaloblastic anemia due to deficiency in vitamin B_{12} is almost inevitable after total gastrectomy if the patient survives the operation for six years or longer, it is rare after operations in which a portion of the stomach remains Recently the authors observed five patients in whom megaloblastic anemia developed after partial gastrectomy and one patient in whom it developed after gastroenterostomy Since intrinsic factor is secreted by the body of the stomach it seemed unlikely that the megaloblastic anemia following partial gastrectomy could be due to lack of this factor However new techniques of investigation made it possible to study such anemias more fully All the patients had a macrocytic anemia with megaloblasts in the sternal marrow They were treated with vitamin B_{12} and four showed optimal response A fifth showed clinical improvement but, although the bone marrow changed from megaloblastic to normoblastic erythropoiesis there was no significant alteration in the peripheral blood a course of folic acid was ineffective but treatment of the accompanying iron deficiency resulted in a good response The sixth patient, whose anemia was mild showed no therapeutic response to either vitamin B_{12} or folic acid The interval between operation and the diagnosis of megaloblastic anemia ranged from 2 to 17 years In all instances the total duration of dyspepsia before and after operation had been 20 years or longer The authors investigated the excretion of acid and uropepsin, and the absorption and excretion of fat They also attempted to obtain biopsy specimens from the remaining portion of the stomach and estimated the vitamin B_{12} content of the serum and the secretion of intrinsic factor The results of the studies indicated that in five of the subjects the anemia was caused by vitamin B_{12} deficiency There was failure to absorb vitamin B_{12} which was corrected by the addition of intrinsic factor the biopsy studies revealed atrophy of the gastric body mucosa comparable in severity to that seen in pernicious anemia In the sixth patient the nature of the deficiency and its pathogenesis remained obscure Steatorrhea was not a significant factor in any of the cases Since megaloblastic anemia is rare after partial gastrectomy and gastroenterostomy it seemed possible that the patients might have Addisonian pernicious anemia and that the gastric operation was only incidental This hypothesis was not tenable in view of the absence of a family history of pernicious anemia and because the histological study of the stomach removed at partial gastrectomy showed normal gastric body glands and in no way resembled the mucosal atrophy seen in pernicious anemia The biopsy

studies did not allow a detailed comparison between the stomach in this condition and in idiopathic pernicious anemia but the authors gained the impression that gastritis was present and they feel that chronic gastritis associated with prolonged dyspepsia gastric ulceration and operative intervention may have contributed to the development of gastric atrophy

92 MacLean, L. D The differentiation of achylia gastrica and achlorhydria by means of radioactive vitamin B_{12} Gastroenterology 29 653 665 Oct 1955

The vitamin B_{12} excretion test differentiates patients with simple achlorhydria and achylia gastrica The latter are possibly more likely to develop gastric cancer Pernicious anemia patients develop gastric cancer more commonly than those who do not have pernicious anemia

This study showed that gastrectomized patients lack the intrinsic factor necessary for absorption of oral vitamin B_{12} A follow up study of patients who survived for over three years after total gastrectomy without vitamin B_{12} or liver extract therapy has shown that 90 per cent develop megaloblastic marrow Apparently pernicious anemia is a late but an inevitable sequela of total or proximal subtotal gastrectomy but is not likely to occur until at least 3 years after the operation

University of Minnesota
Minneapolis Minn.

93 MacLean L D and Sundberg R D Incidence of megaloblastic anemia after total gastrectomy New England J Med 254 885-893 May 10 1956

The authors review of 17 cases of megaloblastic anemia following total gastrectomy reported in the literature suggests that if the patient survives long enough megaloblastic anemia will occur

The authors state that 10 of 11 totally gastrectomized patients (observed by them) who did not receive prophylactic therapy for pernicious anemia and who survived for over three years developed a megaloblastic bone marrow Megaloblastosis occurred irrespective of the type of enteric reconstruction and was not due to blind loop anemia

Six patients with total gastrectomy and 3 with proximal subtotal gastrectomy were unable to absorb oral Co^{60} vitamin B_{12} until intrinsic factor was added This was evidence that the site of intrinsic factor production is the body and fundus the area of pathologic change in the stomach in pernicious anemia The human antrum apparently is not important in elaboration of intrinsic factor

Vitamin B_{12} or purified liver extract alone produced remissions in these patients The authors state that all patients subjected to total gastrectomy or proximal subtotal gastrectomy with esophagoantrostomy should receive continuous prophylactic parenteral vitamin B_{12} or liver extract therapy commencing within three years after operation

U i ersity of Minnesota Medical School
Minneapolis Minn

94 Harvey J C The vitamin B_{12} deficiency state engendered by total gastrectomy Surgery 40 977 989 Nov 1956 (in Recent Advances in Surgery)

In 30 patients treatment with liver extracts or vitamin B_{12} was purposely withheld after total gastrectomy in order to observe the development of vitamin B_{12} deficiency Of the 30 patients 10 were alive at the time of writing their post operative survival time ranging between 2½ years and 17 years

The first evidence of vitamin B_{12} deficiency was macrocytosis of erythrocytes which occurred from six months to seven years (average two and one half years) after gastrectomy Anemia appeared next within an average of four years postoperatively and megaloblasts invariably were found in the marrow within one year after that Three patients who failed to come in for observation according to in

structions had combined nervous system disease six seven and eight years respectively after operation

Parenteral vitamin B_{12} therapy was given to 12 patients whose hematologic picture indicated vitamin B_{12} deficiency All these patients had good hematologic and neurologic responses The author recommends that every totally gastrectomized patient be given parenteral vitamin B_{12} regularly

Johns Hopkins University and Hospital
Baltimore Md

95 Halsted J A, Briggs J D and Gasster M Nutritional problems after total gastrectomy, New York State J Med 57 223-231, Jan 15, 1957

Of 52 totally gastrectomized patients 34 died (of recurrent carcinoma) and 18 survived of whom 17 were available for study six months to nine years postoperatively

Of the 17 patients 12 showed evidence of mild hypochromic anemia due to iron deficiency Since none showed evidence of blood loss it is presumed that an iron absorption defect may exist after gastrectomy and that anemic patients may have lost considerable blood before operation Ferrous sulfate given to 4 patients produced a rise in hemoglobin

Of 9 patients who received no prophylactic therapy megaloblastic anemia due to vitamin B_{12} deficiency developed in only 1 becoming marked five years after operation Therapy with parenteral vitamin B_{12} produced an excellent hematologic response Megaloblastic anemia did not develop in the 7 patients receiving prophylactic vitamin B_{12} liver extract or folic acid therapy

In 12 patients undergoing radioactive vitamin B_{12} absorption tests 80 to 100 per cent of the orally administered radioactivity was recovered indicating little or no absorption of the vitamin When the test dose was mixed with 50 cc of normal human gastric juice most of the Co^{60} vitamin B_{12} was absorbed and in 7 patients absorption was greater than normal The authors conclude that the site of origin of intrinsic factor in man is limited to the stomach '

The small incidence of pernicious anemia following total gastrectomy is explained as follows (1) the daily requirement of vitamin B_{12} is small (1 mcg) and the liver contains approximately a three year supply Thus megaloblastic anemia rarely occurs until at least three years after operation and few patients survive that long In some cases a section of stomach which secretes intrinsic factor is not removed in others, liver extract or vitamin B_{12} is administered

The authors state Patients who have had a total gastrectomy should receive vitamin B_{12} indefinitely to prevent the development of B_{12} deficiency with megaloblastic anemia It must be given parenterally since it cannot be absorbed after removal of the stomach However oral preparations containing intrinsic factor concentrates with vitamin B_{12} should also be effective An appropriate schedule of parenteral administration is 30 to 50 micrograms once a month

University of California Medical School and
Veterans Administration Hospital
Los Angeles Calif

INTESTINAL ANOMALIES

96 Pearson R D Macrocytic anemia associated with intestinal strictures and anastomoses Ann Int Med 40 600-610 March 1954

Case histories of 2 patients with macrocytic anemia associated with intestinal strictures and anastomoses are presented One showed a submaximal response to folic acid and then to intramuscular vitamin B_{12} the other responded well to intramuscular vitamin B_{12} The mechanism by which intestinal strictures or anastomoses cause macrocytic anemia is un

known Removal or short circuiting of considerable lengths of small intestine may result in inadequate absorption of hemopoietic substances or stagnation of intestinal contents may cause changes in bacterial flora which interfere with formation or utilization of hemopoietic substances

Veterans Administration Hospital Bronx N Y

97 Doscherholmen A and Hagen P S Absorption of Co^{60} labeled vitamin B_{12} in intestinal blind loop megaloblastic anemia J Lab & Clin Med 44 790 Nov 1954 (in Soc. Proc)

A laborer was admitted to the hospital in 1952 with a history of progressive weakness intermittent glossitis and numbness of the hands and feet of 6 months duration In 1944 an ileocolostomy for terminal ileitis had been followed by a second laparotomy with closure of the loop of ileum distal to the anastomosis Gradual recovery ensued although he had 4 to 6 loose stools daily On admission blood examination revealed a macrocytic anemia and a megaloblastic bone marrow Gastric juice contained free hydrochloric acid Fecal fat and nitrogen studies were normal X ray examination revealed an ileocolostomy with a blind loop of distal ileum and evidence of ileitis Vitamin C gave suboptimal response while all blood values returned to and remained normal with continued administration of vitamin B_{12} intramuscularly In 1954 physical and laboratory findings were normal but x ray examination revealed progression of the ileitis Absorption studies with 0 5 mcg Co^{60} vitamin B_{12} given orally resulted in fecal excretion of 78 per cent no urinary radioactivity followed a flushing intramuscular dose of 1 000 mcg of vitamin B_{12}

The addition of up to 300 mg of intrinsic factor concentrate pretest saturation with oral vitamin B_{12} and attempted sterilization of the gut with neomycin failed to cause normal vitamin B_{12} absorption The patient s gastric juice however effected some absorption of Co^{60} vitamin B_{12} in a patient with pernicious anemia

Minneapolis Minn

98 Badenoch J Bedford P D and Evans J R Massive diverticulosis of the small intestine with steatorrhoea and megaloblastic anaemia Quart J Med 24 321 330 1955 (abstr Blood 11 1053 1054 Nov 1956)

Three cases of steatorrhea with megaloblastic anemia have now been described in association with massive diverticulosis of the small intestine Additional observations are reported on one of these cases and three others are described In one of the four cases diverticulosis was not suspected but was found post mortem in the others the condition was demonstrated by barium meals In two the absorption of labeled cyanocobalamin was investigated and found to be grossly impaired but this defect was not completely corrected by the addition of the small amount of intrinsic factor which is effective in pernicious anemia A further improvement occurred when larger amounts of intrinsic factor were given as occurs in those cases of idiopathic steatorrhea where megaloblastic anemia is associated with malabsorption of vitamin B_{12} It is considered that jejunal diverticulosis causes steatorrhea and that the megaloblastic anemia is secondary to this Unlike what occurred in one case of pernicious anemia and one of idiopathic steatorrhea the administration of Aureomycin enhanced the absorption of labeled cyanocobalamin in the two patients with intestinal diverticulosis and megaloblastic anemia who were investigated in this way

Radcliffe Infirmary and Cowley Road Hospital Oxford England

99 Thompson, R B and Ungley, C C Megaloblastic anemia associated with anatomic lesions in the small intestine, Blood 10 771 787, Aug 1955

Authors summary ' This paper describes the development of anemia in six patients with strictures and anastomoses *in the small intestine The marrow proved* to be megaloblastic in three instances and megaloblastic change is presumed in the other three because of the clinical and laboratory findings, and the characteristic hemopoietic response to liver therapy Responses to crude and refined liver extracts [5 cc] and vitamin B_{12} [80 mcg orally and 80 to 160 mcg intramuscularly] compared unfavorably with those to be expected in Addisonian pernicious anemia they were similar to the poorer responses often observed in megaloblastic anemia associated with idiopathic steatorrhea The relationship of the intestinal lesion to the development of megaloblastic anemia is briefly discussed

Royal Victoria Infirmary
Newcastle on Tyne England

100 Halsted, J A , Lewis, P M and Gasster M Absorption of radioactive vitamin B_{12} in the syndrome of megaloblastic anemia associated with intestinal stricture or anastomosis, Am J Med 20 42 52, Jan 1956

The effect of antibiotics and intrinsic factor on the absorption of vitamin B_{12} was studied in 2 patients with megaloblastic anemia associated with intestinal stricture or anastomosis Both patients responded to parenteral vitamin B_{12} but developed anemia when it was discontinued Test doses of 0 5 mcg of Co^{60} vitamin B_{12} were given alone then with intrinsic factor and later immediately following daily administration of 2 Gm of Aureomycin o hromycin or 6 Gm of neomycin d again after 10 to 14 days

Fecal min B_{12} was not decrea n of intrinsic factor greater in 1 patient Both Aureomycin and Achromycin produced markedly increased absorption of vitamin B_{12} but neomycin had no effect Since Achromycin and Aureomycin (but not neomycin) also decreased diarrhea in 1 patient it was thought that the increased absorption of vitamin B_{12} might be due to this effect rather than to the direct effects of the antibiotics A test was therefore done using Gantrisin which completely cured the diarrhea but caused no decrease in fecal excretion of vitamin B_{12} Thus diarrhea was not the reason for the high excretion of vitamin B_{12} in this patient

The authors in their summary state

These studies provide additional support for the concept that abnormal bacterial growth in the small intestine may result in impaired utilization of vitamin B_{12} with the development of megaloblastic anemia in some instances The exact mechanism whereby intestinal bacteria *may affect hematopoiesis adversely* and certain antibiotics may favorably influence it, is not yet clear

University of California Medical Center
Los Angeles Calif

SPRUE AND STEATORRHEA

101 Glass, G B J Intestinal absorption and hepatic uptake of vitamin B_{12} in diseases of the gastrointestinal tract, Gastroenterology 30 37 52 Jan 1956

Absorption of vitamin B_{12} was studied by giving patients an oral tracer dose of 0 5 m of Co^{60} vitamin B_{12} and measur in uptake of the vitamin by , ting of the liver a orption of vita e but not all idity in elder cidity or a omized p all these mitant a

hepa rpti

min B_{12} in 10 patients with intestinal disease The absorption in 4 patients with irritable colon was good In 4 with sprue and in 2 with regional ileitis no hepatic uptake of oral vitamin B_{12} occurred and this could not be corrected by giving intrinsic factor

The author states that in the future determination of the intestinal absorption of vitamin B_{12} by the isotope technic probably will permit determination of the extent of gastric atrophy This would be helpful in detecting the precursors of pernicious anemia among the apparently healthy persons and would aid in differentiating pernicious anemia from other macrocytic anemias The test also permits the detection of a sprue-like absorption pattern

New York Medical College and
Flower and Fifth Avenue Hospitals
New York N Y

102 Meynell M J Cooke W T Cox E V and Gaddie R. Serum-cyanocobalamin level in chronic intestinal disorders Lancet 1 901 904 May 4 1957

A method is presented for the assay of vitamin B_{12} in sera using *L. leichmannii* Serum levels were studied in 78 patients with idiopathic steatorrhea in 43 with regional ileitis in 90 control hospital patients with various diseases and in 43 healthy volunteers

Ninety six per cent of all controls had levels within the normal range (100 to 450 micromicrograms per cc) The 2 control patients with levels below normal had peptic ulcers Of 3 controls with levels above normal 1 had congestive cardiac failure and hepatomegaly

In 22 of 78 patients with idiopathic steatorrhea the serum cyanocobalamin levels were below 100 micromicrograms per cc and in 14 others a deficiency was diagnosed from the hematologic response to vitamin B_{12} therapy

Of 43 patients with regional ileitis 23 had abnormally low serum cyanocobalamin levels and 3 others were being treated with cyanocobalamin for megaloblastic anemia Most patients with regional ileitis had free hydrochloric acid in the stomach but some had loss of weight energy and concentration irritability personality changes and occasional psychoses which cleared during vitamin B_{12} therapy Most patients with steatorrhea or ileitis had no hematologic manifestations

The serum cyanocobalamin levels in idiopathic steatorrhea and in regional ileitis contrast with the normal levels found in enterocolitis in ulcerative colitis and even more strikingly with the high levels (280 to 1 250 micromicrograms per cc) found in cirrhosis

General Hospital
Birmingham, England

103 Oxenhorn S , Estren, S and Adlersberg D Intestinal uptake of vitamin B_{12} in the malabsorption syndrome, J Mt Sinai Hosp 24 232 242 May June 1957

A modification of Schilling s urinary excretion test was employed in the study of vitamin B_{12} absorption in pernicious anemia and the malabsorption syndrome Severely defective vitamin B_{12} absorption was noted in all patients with pernicious anemia This defect was fully corrected by the addition of intrinsic factor Deficient vitamin B_{12} absorption was also noted in 20 of 25 patients with idiopathic sprue and in 3 with a sprue syndrome secondary to the surgical removal of most of the small intestine Intrinsic factor failed to improve vitamin B_{12} absorption in either of these groups

The Mount Sinai Hospital
New Yo k N Y

104 Estren S The blood and bone marrow in idiopathic sprue J Mt Sinai Hosp 24 304-316 May June 1957

There are multiple intestinal defects of absorption in idiopathic sprue Those of importance in the development of anemia include malabsorption of dietary protein folic acid vitamin B_{12} and iron Re-

99 Thompson, R B and Ungley, C C Megaloblastic anemia associated with anatomic lesions in the small intestine, Blood 10 771-787, Aug 1955

Authors summary This paper describes the development of anemia in six patients with strictures and anastomoses in the small intestine The marrow proved to be megaloblastic in three instances, and megaloblastic change is presumed in the other three because of the clinical and laboratory findings and the characteristic hemopoietic response to liver therapy Responses to crude and refined liver extracts [5 cc] and vitamin B_{12} [80 mcg orally and 80 to 160 mcg intramuscularly] compared unfavorably with those to be expected in Addisonian pernicious anemia they were similar to the poorer responses often observed in megaloblastic anemia associated with idiopathic steatorrhea The relationship of the intestinal lesion to the development of megaloblastic anemia is briefly discussed

Royal Victoria Infirmary
Newcastle on Tyne England

100 Halsted, J A, Lewis P M and Gasster M Absorption of radioactive vitamin B_{12} in the syndrome of megaloblastic anemia associated with intestinal stricture or anastomosis, Am J Med 20 42 52 Jan 1956

The effect of antibiotics and intrinsic factor on the absorption of vitamin B_{12} was studied in 2 patients with megaloblastic anemia associated with intestinal stricture or anastomosis Both patients responded to parenteral vitamin B_{12} but developed anemia when it was discontinued Test doses of 0 5 mcg of Co^{60} vitamin B_{12} were given alone then with intrinsic factor and later immediately following daily administration of 2 Gm of Aureomycin or Achromycin or 6 Gm of neomycin for 3 days and again after 10 to 14 days

Fecal excretion of vitamin B_{12} was not decreased by administration of intrinsic factor Excretion was even greater in 1 patient Both Aureomycin and Achromycin produced markedly increased absorption of vitamin B_{12} but neomycin had no effect Since Achromycin and Aureomycin (but not neomycin) also decreased diarrhea in 1 patient, it was thought that the increased absorption of vitamin B_{12} might be due to this effect rather than to the direct effects of the antibiotics A test was therefore done using Gantrisin which completely cured the diarrhea but caused no decrease in fecal excretion of vitamin B_{12} Thus diarrhea was not the reason for the high excretion of vitamin B_{12} in this patient

The authors in their summary state

These studies provide additional support for the concept that abnormal bacterial growth in the small intestine may result in impaired utilization of vitamin B_{12} with the development of megaloblastic anemia in some instances The exact mechanism whereby intestinal bacteria may affect hematopoiesis adversely and certain antibiotics may favorably influence it is not yet clear

University of California Medical Center
Los Angeles Calif

SPRUE AND STEATORRHEA

101 Glass, G B J Intestinal absorption and hepatic uptake of vitamin B_{12} in diseases of the gastrointestinal tract Gastroenterology 30 37 52 Jan 1956

Absorption of vitamin B_{12} was studied by giving patients an oral tracer dose of 0 5 mcg of Co^{60} vitamin B_{12} and measuring the hepatic uptake of the vitamin by scintillation counting of the liver area

Impaired absorption of vitamin B_{12} was found in some but not all patients with gastric anacidity in elderly patients with gastric hypoacidity or anacidity and in partially gastrectomized patients Absorption levels in all these patients were increased by concomitant administration of intrinsic factor

Measurement of hepatic uptake was also used to study absorption of oral vita

vitamin B_{12} to a specific portion of gastric juice by paper electrophoresis may be a clue to the further identification of the intrinsic factor of Castle

University of Wisconsin Medical School
Madison, Wis

109 Council on Pharmacy and Chemistry Vitamin B_{12} with intrinsic factor concentrate J A M A 153 565 Oct 10 1953

Vitamin B_{12} also known as the extrinsic factor when combined with intrinsic factor concentrate is effective orally for the treatment of pernicious anemia with and without neurological complications It is also effective by virtue of its vitamin B_{12} content in the treatment of tropical and nontropical sprue nutritional macrocytic anemia caused by vitamin B_{12} deficiency and macrocytic anemia of infancy

Oral vitamin B_{12} with intrinsic factor concentrate is suitable to replace injectable cyanocobalamin or liver for [pernicious anemia] patients in whom parenteral therapy is difficult or undesirable Patients should be carefully observed during oral treatment if expected improvement does not occur further examination should be made to rule out complicating disorders such as infection gastrointestinal malfunction or undiagnosed malignant disease In the presence of any such complication the dosage may need to be increased or abandoned in favor of injection therapy with cyanocobalamin or liver

The potency is expressed in terms of the U.S P oral unit of hematopoietic activity The average daily dosage for the treatment of pernicious and related macrocytic anemias is 1 U S P oral unit daily

110 Schilling R.F A new test for intrinsic factor activity J Lab & Clin Med 42 946-947 Dec 1953 (in Soc Proc)

Progress toward the isolation of intrinsic factor has been hampered by the necessity of a tedious bioassay in patients with pernicious anemia in relapse As part of a new test for intrinsic factor activity 6 normal persons and 10 pernicious anemia patients in remission were each given 2 mcg of radioactive vitamin B_{12} orally and two hours later 1 000 mcg of nonradioactive vitamin B_{12} by subcutaneous injection Within four to six hours after the oral dose radioactivity appeared in the urine of the normals persisting for at least 20 hours but it did not appear in urine of the pernicious anemia patients When the test was repeated in pernicious anemia patients with addition of human gastric juice to the radioactive vitamin B_{12} they all excreted radioactivity in the urine Boiling the gastric juice destroyed its ability to produce this effect as did giving it 12 hours after the oral Co^{60} vitamin B_{12} dose Two preparations of hog duodenum with intrinsic factor activity also produced radioactivity in the urine of pernicious anemia patients when given simultaneously with the oral Co^{60} vitamin B_{12}

This test can be done repeatedly on pernicious anemia patients in complete remission The limiting factor is the safe dose of Co^{60}

Madison, Wis

111 Callender S T, Turnbull A and Wakisaka G Estimation of intrinsic factor of Castle by use of radioactive vitamin B_{12} Brit M J 1 10-13 Jan 2 1954

Absorption of vitamin B_{12} has been assessed by measuring fecal radioactivity after an oral dose of 0 5 mcg of Co^{60} vitamin B_{12} In 10 normal subjects a mean of 31 0 per cent (standard deviation 6 9 per cent) of the radioactivity was recovered In 13 patients with pernicious anemia a mean of 88 7 per cent (standard deviation 6 8 per cent) was recovered When intrinsic factor was given with the radioactive vitamin B_{12} to 8 patients with pernicious anemia the percentage recovered was reduced greatly

Radcliffe Infirmary
Oxford England

112 Yamamoto, R S and Chow, B F A rapid method for the determination of B_{12} binding power in the gastric juice J Lab & Clin Med 43 316-320, Feb 1954

Authors summary and conclusion Vitamin B_{12} combines with one or more substances in gastric juice in such a manner as to make it unadsorbable by resting cells of *L leichmannii* This fact enables the development of a method for the measurement of the binding power of the gastric juice With this procedure it was demonstrated that specimens obtained from pernicious anemia patients and from old individuals without achlorhydria had significantly lower binding power than control specimens Gastric juice specimens from three cases of juvenile pernicious anemia and from old people with achlorhydria contained at least as much [binding power] as the normal samples The importance of such findings is discussed

School of Hygiene and Public Health
Johns Hopkins University
Baltimore Md

113 Glass G B J Uptake of radioactive vitamin B_{12} by the liver test for intestinal absorption of vitamin B_{12} and measure of the intrinsic factor activity Clin Research Proc 2 32, March 1954

By scintillation measurement of the hepatic uptake of ingested Co^{60} vitamin B_{12} in 8 controls and 8 patients with macrocytic anemias a test procedure was developed for quantitation of intestinal utilization of vitamin B_{12} in humans and measurement of intrinsic factor activity In nutritional and hemolytic anemias with normal gastric secretion hepatic uptake of ingested radioactive vitamin B_{12} was normal, but in 3 patients with pernicious anemia in relapse and remission it was negligible or nil This indicated defective transport of ingested vitamin B_{12} to the liver in pernicious anemia The liver uptake became normal after the same dose of Co^{60} vitamin B_{12} was taken with normal human gastric juice Thus the ability of normal gastric juice to promote the transfer of vitamin B_{12} through intestinal wall to the liver was directly demonstrated the reason for intestinal block to utilization of vitamin B_{12} in pernicious anemia patients is the absence of intrinsic factor from their gastric juice

In sprue hepatic uptake of vitamin B_{12} was also negligible, but it was not corrected by addition of normal human gastric juice or intrinsic factor concentrate This indicated that the absorption defect in sprue was not caused by lack of intrinsic factor in the gastric juice but by a defect in the intestinal wall itself

New York Medical College
New York N Y

114 Schilling, R F Recent studies of intrinsic factor and the utilization of radioactive vitamin B_{12}, Federation Proc 13 769 775, Sept 1954

The author reviews the methods employing radioactive vitamin B_{12} that have been used to corroborate the fact that intrinsic factor enhances the utilization of orally administered vitamin B_{12} After discussing the chemical nature of the intrinsic factor the conclusion is that although intrinsic factor is a mucoprotein not all mucoprotein in gastric juice is intrinsic factor

In summary it is stated that intrinsic factor probably exerts a unique type of biological activity for no other substances are known which are secreted exclusively to enhance absorption of a specific vitamin It is suggested however that there may be intrinsic factors for other essential nutrients

University of Wisconsin Medical School
Madison Wis

115 Baker S J and Mollin D L The relationship between intrinsic factor and the intestinal absorption of vitamin B_{12} Brit J Haemat 1 46-51, 1955 (abstr Am J Clin Path. 25 1200 Oct 1955)

Vitamin B_{12} labeled with Co^{58} (half life 72 days) was given to patients with

pernicious anemia in remission and fecal radioactivity was measured Over a certain range there was a stoichiometric relationship between the amount of vitamin B_{12} absorbed and the amount of intrinsic factor administered Beyond this range markedly increased amounts of intrinsic factor produced no increased absorption of the vitamin Individuals varied in ability to absorb vitamin B_{12} administered with a given amount of intrinsic factor Such variations suggest that the development of vitamin B_{12} deficiency depends not only on failure of intrinsic factor production but also on inability to absorb vitamin B_{12} intrinsic factor complex

116 Glass G B J Boyd L J Stephanson L and Jones E L Metabolic interrelations between intrinsic factor and vitamin B_{12} III B_{12} absorption at varied intrinsic factor doses Proc Soc. Exper Biol & Med 88 1 5 Jan. 1955

The authors administered varying dosages of intrinsic factor with constant dosage of Co^{60} vitamin B_{12} to 8 patients with pernicious anemia in remission to 1 patient with total gastrectomy and to 1 control

Up to a certain point increasing the oral dosage of intrinsic factor while the dosage of vitamin B_{12} remained constant increased the intestinal absorption of the vitamin, as measured by the hepatic uptake of Co^{60} vitamin B_{12} Further increase in the dosage of intrinsic factor however may decrease intestinal absorption of vitamin B_{12} Unless the dosage ratio of vitamin B_{12} to intrinsic factor is optimal results of assays to determine intrinsic factor potency may be erroneous

New York Medical College
Flower and Fifth A enue Hospitals
N w Yo k N Y

117 Rabiner S F Fllenbogen L, Lichtman H C Williams W Kabakow B and Watson R J Urinary excretion of vitamin B_{12} Co^{60} as a method of assay for intrinsic factor Clin Research Proc 3 28 29 Feb 1955

I atients with pernicious anemia in remission were given orally 2 mcg of Co^{60} vitamin B_{12} and simultaneously injected intramuscularly with 1 0 to 4 0 mg of unlabeled vitamin B_{12} Urine collected for the next 24 hours was measured for radioactivity Subsequently animal intrinsic factor was fed to the patient along with the radioactive vitamin B_{12} and the injected nonradioactive vitamin B_{12} Little or no radioactivity was found in the first 24 hours in the urine of patients with pernicious anemia Under the same conditions normal persons excreted 5 to 20 per cent (average 11 per cent) of the radioactive dose administered When patients with pernicious anemia were given the clinically active source of intrinsic factor however their average urinary excretion values were approximately normal Intrinsic factor preparations of submaximal clinical activity produced urinary excretion values in such patients between 1 and 5 per cent The data indicate that the assay of intrinsic factor can be performed rapidly and accurately using patients with pernicious anemia in remission

St te University of New York College of Medicine
New York N Y
Lederle Laboratories
Pea l River N Y

118 Ellenbogen L, Williams W L Rabiner S F and Lichtman H C An improved urinary excretion test as an assay for intrinsic factor Proc Soc Exper Biol & Med 89 357 362 July 1955

A procedure is suggested for assaying intrinsic factor preparations by measuring urinary radioactivity following oral dosage with Co^{60} vitamin B_{12} More than one intramuscular flushing dose of unlabeled vitamin B_{12} is given to produce maximum excretion of radioactivity With this method the same patient can be used for assaying several preparations of intrinsic factor in succession The injection

of unlabeled vitamin B_{12} in doses several times as large as the usual flushing dose appeared to decrease the uptake of subsequent small oral doses of labeled vitamin B_{12} for reasons not fully understood There appeared to be some correlation between the dosage of intrinsic factor and the amount of radioactivity excreted Patients with pernicious anemia and normal persons were tested

American Cyanamid Co and Lederle Laboratories Pearl River N Y

State University of New York College of Medicine New York N Y

119 Bishop, R C, Toporek M, Nelson, N A. and Bethell F H The relationship of binding power to intrinsic factor activity, J Lab & Clin Med 46 796, Nov 1955 (in Soc Proc)

'The role of intrinsic factor in the absorption of vitamin B_{12} has long been under investigation Some substances with intrinsic factor activity bind B_{12} and make it unavailable for growth in the microbiologic assay for the vitamin The relationship of this binding power to intrinsic factor activity has not yet been definitely established When pernicious anemia (PA) patients were used to evaluate the intrinsic factor activity of experimental preparations there was an incomplete correlation between such activity and the binding power of these preparations One of the difficulties presented by the use of PA patients is the fact that the gastric juice of such patients does have some binding power

To circumvent the possible effect of the binding power of the PA patients own gastric juice the binding power intrinsic factor relationship was studied in patients with total gastrectomies using the urinary excretion of orally administered radioactive B_{12} Co^{60} as a measure of absorption A control test oral B_{12} Co^{60} without intrinsic factor was followed by other tests at weekly intervals In one an oral dose of B_{12} Co^{60} was followed immediately by an oral dose of normal human gastric juice (GJ) which had been incubated for 30 minutes at room temperature with equivalent amount of nonradioactive B_{12} In the second part of the experiment, the B_{12} Co^{60} was bound to GJ and the nonradioactive B_{12} was free As a check on the effect of prolonged contact apart from binding on absorption a test was run with B_{12} Co^{60} and gastric juice administered separately without prior incubation The findings confirmed earlier observations that prior incubation does not enhance absorption

' The data indicate that the B_{12} already bound by the GJ is preferentially absorbed One series of tests on a PA patient gave similar results It is concluded that binding power is necessary for intrinsic factor activity

Ann Arbor Mich

120 Bull, F E, Campbell, D C and Owen C A, Jr Evaluation of intrinsic factor in pernicious anemia by means of cobalt 60-vitamin B_{12} Federation Proc 15 509 510, March 1956

Absorption of orally administered Co^{60} vitamin B_{12} may be estimated by measurement of (1) radioactivity in feces (2) radioactivity in the liver or (3) radioactivity in urine after a flushing dose of parenterally administered unlabeled vitamin B_{12} These methods were used to study intrinsic factor activity in 92 patients A sharp distinction in absorption of vitamin B_{12} was evident by all techniques when normals were compared to patients with pernicious anemia The urinary method was simplest and quickest but unrecognized loss of part of the specimen might suggest pernicious anemia in a normal person In patients with uremia renal excretion of Co^{60} often was so retarded that a single 24 hour urinalysis gave misleadingly low results however total excretion over a period of several days was normal The fecal-excretion method was accurate but laborious here unrecognized loss of specimens might

yield false normal values Hepatic radio activity was measurable only after unabsorbed intestinal Co^{60} was excreted this method was slow but perhaps most accurate since it did not depend on the patient s cooperation in collecting excreta When initial tests indicated lack of intrinsic factor they were repeated with the addition of gastric juice to the labeled vitamin patients with pernicious anemia then showed normal absorption but patients with severe sprue or intestinal disease often did not

Mayo Foundation and Mayo Clinic Rochester Minn

121 Best W R White W F Robbins K C Landmann W A and Steelman S L. Studies on urinary excretion of vitamin B_{12} Co^{60} in pernicious anemia for determining effective dosage of intrinsic factor concentrates Blood 11 338-351 April 1956

In order to assay intrinsic factor concentrates and to determine optimal dosage schedules for oral therapy urinary excretion of Co^{60} vitamin B_{12} was determined in patients with pernicious anemia Co^{60} vitamin B_{12} was given orally with intrinsic factor and followed by a flushing parenteral dose Assay of intrinsic factor by this method is valid only if the ratio of intrinsic factor to inactive vitamin B_{12} binding material is reasonably constant and if the intrinsic factor contains insignificant amounts of vitamin B_{12}

The authors found that increasing doses of intrinsic factor concentrates give increasing excretions of radioactivity with low doses of vitamin B_{12} little additional increase with moderate doses and sometimes a diminution with excessive doses Assay by urinary excretion was compared with assay by hematologic response in 13 patients with pernicious anemia in relapse The two tests showed some correlation but individual values varied greatly The authors state that lack of a pure intrinsic factor and variations in individual patients make it impossible to formulate the exact relationship of intrinsic factor dose vitamin B_{12} dose and intestinal absorption of vitamin B_{12}

University of Illinois College of Medicine Chicago Ill

122 Johnson P C Richmond V, Caputto R and Wolf S Fractionation of human gastric content the separation of intrinsic factor activity J Clin Investigation 35 716 June 1956 (in Soc Proc)

A method of identification of intrinsic factor in material from the fractionation of human gastric contents (chromatography) was studied in 4 patients with peptic ulcer in 4 with pernicious anemia and in healthy subjects In patients with peptic ulcer and in normal subjects seven liters of effluent yielded 50 to 75 per cent recovery The first liter of effluent contained the major portion of the protein and carbohydrate in two well defined peaks Only the fourth liter contained intrinsic factor activity however and here the zone of activity was narrowed down to a single 400 cc fraction From this fraction as little as 10 mg of dried material produced absorption of the tagged vitamin B_{12} in subjects in whom no absorption had been demonstrated in control runs These experiments provide the first step toward the establishment of a normal fractional pattern of human gastric content Such a pattern would not only allow for the direct recognition of missing biologically active components but like the fractions of blood serum might ultimately be useful in differential diagnosis

Oklahoma City Okla

123 Chow B F Quattlebaum J K Jr and Rosenblum C Effect of intrinsic factor concentrate on vitamin B_{12} absorption by gastrectomized rats Proc Soc Exper Biol & Med 90 279 281, Oct 1955

Radioactive vitamin B_{12} with and without exogenous intrinsic factor was

administered orally to totally gastrectomized rats Absorption of vitamin B_{12} was measured by scintillation counting of the feces No absorption occurred in the gastrectomized rats whether or not intrinsic factor was given Intrinsic factor inhibited the absorption of vitamin B_{12} by control rats

The authors suggest that rats can utilize only endogenous intrinsic factor and that exogenous intrinsic factor is ineffective or inhibitory The studies may also indicate that the stomach is the major site of vitamin B_{12} absorption by the rat Studies of the same type will be made using rat stomach concentrate If this concentrate proves effective it will demonstrate species specificity of intrinsic factor

On a weight basis the rat appears capable of absorbing greater quantities of vitamin B_{12} than the human Thus in the absence of intrinsic factor absorption from an oral dose of 50 micromicrograms amounts to 20 to 25 micromicrograms and from 200 micromicrograms absorption is 30 micromicrograms The equivalent oral doses for a 60 Kg human would be 10 mcg and 40 mcg and the corresponding absorption 4 to 6 mcg Actually the human absorbs only 1 5 mcg from such doses

Johns Hopkins University
Baltimore Md
Merck & Co
Rahway N J

124 Nieweg, H O Arends, A , Mandema, E and Castle W B Enhanced absorption of vitamin B_{12} in gastrectomized rat by rat intrinsic factor, Proc Soc Exper Biol & Med 91 328 332 Feb 1956

In preliminary studies to develop an animal preparation for the detection of intrinsic factor, male rats were totally gastrectomized and anastomosis of the esophagus to the pylorus was done Rats in good health 10 days after the operation were fed a low residue synthetic diet containing all essential nutrients including vitamin B_{12} Radioactive vitamin B_{12} 0 015 mcg in 1 cc of water, and intrinsic factor preparations were given by stomach tube Feces assayed for vitamin B_{12} showed that normal rats absorbed a significant amount while gastrectomized rats absorbed none Both normal human gastric juice in doses of 2 to 6 cc , and hog stomach mucosa in doses of 4 to 20 mg were ineffective in improving vitamin B_{12} absorption The authors conclude that assimilation of vitamin B_{12} in the rat in some ways parallels that in man and that the negative results obtained with intrinsic factor preparations from other species may point to species specificity for intrinsic factor preparations

Boston City Hospital
Harvard Medical School
Boston, Mass
University of Groningen
Groningen Holland

LIVER

VITAMIN B_{12} AND LIVER DISEASE

125 Jones P N and Mills E H. Serum vitamin B_{12} concentrations in liver disease J Lab & Clin Med 46 927, Dec 1955 (in Soc Proc)

Serum vitamin B_{12} concentrations were studied in 11 patients with Laennec s cirrhosis in 10 with postnecrotic cirrhosis in 4 with biliary cirrhosis, in 6 with acute viral hepatitis and in 4 in hepatic coma Those with biliary cirrhosis had values within the normal range all others had vitamin B_{12} serum concentrations at least 3 to 8 times normal Patients in hepatic coma had values between 30 and 40 times normal Patients with Laennec s cirrhosis had proportionate rises in both the free

ombined vitamin B_{12} while those
icute hepatitis or postnecrotic cir
had a greater proportional rise in
e serum vitamin B_{12}
eterminations made on 24 hour
collections showed that these pa
excreted from 10 to 30 times the
l amount of vitamin B_{12} all in the
rm
is concluded that the metabolism of
in B_{12} is seriously altered in both
and chronic liver disease

, Ill

s G B J Fbin L M Boyd
and Laughton R Hepatic up-
of radioactive vitamin B_{12} in
disease Federation Proc. 15 74-
March 1956

o evaluate uptake of vitamin B_{12} by
amaged liver 35 measurements with
vitamin B_{12} were performed in 15
nts with diffuse liver disease The
ic uptake was measured by scintilla
ounting the surface of the liver fol
g parenteral or oral administration
^{60} vitamin B_{12} alone or with potent
sic factor preparation The data
obtained at various stages of disease
correlated with liver function tests
he clinical picture The hepatic up
of orally administered Co^{60} vitamin
as temporarily abolished in 1 patient
severe virus hepatitis as well as in 1
severe cirrhosis and in 1 with obstruc
aundice Of 3 others with hepatitis
th cirrhosis and 3 with obstructive
dice the hepatic uptake of Co^{60} vita
B_{12} was at a low level in 6 In 4 out of
es of metastatic or primary liver car
ma however the hepatic uptake was
al both on oral and parenteral
istration
he addition of intrinsic factor prep
on in 8 out of 9 tests did not improve
decreased hepatic uptake of Co^{60}
nin B_{12} This decreased uptake obvi
y does not depend on the decreased
ut of intrinsic factor but on the im
paired ability of the diseased liver to pick up or retain vitamin B_{12} It appears how ever, that an advanced lesion does not always severely impair the liver s ability to anchor vitamin B_{12} especially after parenteral administration

New York Medical College
New York N Y

127 **Stevenson T D and Beard M F Observations on the metabolism of vitamin B_{12} in patients with liver dis ease Clin Research Proc 4 144, April 1956**

Studies on patients with liver disease showed that levels of serum vitamin B_{12} were elevated only in those with hepatic parenchymal disease i e alcoholic cir rhosis and infectious hepatitis and that the serum level of vitamin B_{12} was mark edly elevated in patients in hepatic coma Patients with obstructive jaundice did not show increased serum levels of vitamin B_{12}

Observations indicate that return of the serum vitamin B_{12} level to normal parallels clinical improvement The evi dence suggests that the elevation of the serum level of vitamin B_{12} in patients with liver disease represents the release of the vitamin from hepatic cells

University of Louisville
School of Medicine
Louisville Ky

128 **Rachmilewitz, M, Aronovitch J and Grossowicz N Serum concen trations of vitamin B_{12} in acute and chronic liver disease J Lab & Clin Med 48 339 344 Sept 1956**

High vitamin B_{12} values were found in the sera of 13 of 14 patients with acute viral hepatitis Concentrations ranged from 800 to 6 000 micromicrograms per cc and decreased gradually with subsi dence of jaundice In 5 cases of biliary cirrhosis and in 3 of 6 cases of portal cir rhosis the vitamin B_{12} concentration ranged from 830 to 3 600 micromicro- grams per cc In 6 cases of extrahepatic biliary obstruction and jaundice however

the serum vitamin B_{12} was within normal range

The maximal binding capacity (MBC) of the serum of normal individuals ranged from 1 200 to 2 000 micromicrograms per cc In patients with liver disease and high serum vitamin B_{12} it ranged from 1,000 to 3 800 micromicrograms per cc

The increase of serum vitamin B_{12} in liver disease is considered by the authors to be due to the release of the stored vitamin from damaged liver cells

Hebrew University
Hadassah Medical School
Jerusalem Israel

129 Baker H, Pasher I, Dolger H P and Sobotka, H Vitamin B_{12} excretion as index of hepatic disorder, Clin Chem 2 328 330 Oct 1956

This paper reports the decreased renal excretion of intramuscular vitamin B_{12} in patients with liver involvement, and the gradual increase to normal levels during the period of convalescence Urinary excretion of vitamin B_{12} using *L leichmannii Euglena gracilis* and *Ochromonas malhamensis* assays was determined for 20 patients with liver disease and for 3 controls following a single intramuscular load dose of 50 mcg of vitamin B_{12}

Normal controls excreted 24 to 40 mcg of the 50 mcg dose while patients with liver disease excreted 10 mcg or less Some patients considered convalescent also failed to excrete normal amounts of vitamin B_{12} Four patients recovering from pneumonia also excreted less than 10 mcg of vitamin B_{12} indicating the effect of the infection on the liver

It is stated that a high excretion of vitamin B_{12} after a load dose would indicate that the liver was again capable of storing the vitamin and that increased amounts from injection are no longer required for normal metabolic activity of the organ

The various liver disorders are being categorized by determining the extent to which vitamin B_{12} excretion is correlated with liver damage This may aid in detecting how extensive the liver damage is and how far convalescence has progressed

Mount Sinai Hospital
New York N Y

130 Ostergaard Kristensen H P The vitamin B_{12} concentration in plasma from patients with acute hepatitis and obstructive jaundice, Nord med 55 85 87, 1956 (abstr Blood 11 1055 1056 Nov 1956)

The plasma concentration of vitamin B_{12} was determined microbiologically in patients with acute hepatitis obstructive jaundice and hepatic cirrhosis *Lactobacillus leichmannii 313* was used as the test organism In the active phase of acute hepatitis a remarkable increase in plasma vitamin B_{12} activity was found In obstructive jaundice and hepatic cirrhosis normal or slightly increased values were found

University of Copenhagen
Biochemical Institute
Copenhagen Denmark

131 Posteraro P The treatment of minor hepatic dysfunction in children, Minerva pediat 6 32 Jan 15 1954 (abstr A M A Am J Dis Child 91 399, April 1956)

Signs of minor hepatic dysfunction in children may often follow infection where sulfonamides or antibiotics have been given The symptoms may include pallor subicteric sclerae slightly enlarged liver asthenia, anorexia fat indigestion and urticaria The author has treated 50 children with this condition with very favorable results by a preparation containing betaine choline and vitamins B_1 and B_{12}

132 Canivell F Viral hepatitis some pediatric aspects Arch pediat Barcelona 4 379 387 Jan -Feb 1954 (abstr A M A Am J Dis Child 89 753 June 1955)

The writer discusses the epidemiology symptomatology diagnosis and differen

tial diagnosis of viral hepatitis Importance of test is emphasized Chlortetracycline and oxytetracycline and choline and methionine are described as effective but must not be given in too large doses Vitamin B_{12} folic acid yeast and a diet high in protein and carbohydrates but exceedingly poor in fats should be maintained as long as needed

133 Colwell A R Jr Occurrence of accumulation of fat in the liver and its relation to excess weight gain in patients convalescing from viral hepatitis Ann Int Med 41 963 979 Nov 1954

In 144 soldiers convalescing from viral hepatitis liver biopsy showed fat accumulation in the liver of about half Those showing liver fat gained more than twice as much body weight during convalescence as those showing no abnormal fat in the liver The amount of liver fat in these hepatitis patients did not approach that seen in alcoholic cirrhosis Fat deposition never occurred in the acute phase of hepatitis but was seen 6 to 13 weeks after onset of the disease

About 60 patients had been given vitamin B_{12} 30 mcg every other day (apparently intramuscularly) during the very early phases of hepatitis 8 received 15 mcg every other day for four weeks This failed to prevent abnormal accumulation of liver fat and did not shorten convalescence The author says however that use of vitamin B_{12} might be justified to increase appetite

The author believes that increased intake of calories and fat and perhaps a relative protein deficiency were responsible for the fat deposition Hepatic function as estimated by the bromsulphalein retention test was not impaired by the accumulation of fat and the incidence of reticulinosis was not increased Although it is impossible to state whether this fat is harmful its presence on histologic section is abnormal Therefore the dietary fat and caloric intake should be moderately limited so that obesity does not occur

U S A my Hospital
Kyoto Japan

134 Campbell R E and Pruitt F W The effect of vitamin B_{12} and folic acid in the treatment of viral hepatitis Am J M Sc 229 8 15 Jan 1955

A control group of 44 patients with acute viral hepatitis received conventional treatment consisting of high protein high carbohydrate moderate fat diet and bed rest Another group of 44 patients comparable in race age duration of illness and degree of icterus received the same treatment plus 30 mcg of vitamin B_{12} by intramuscular injections every other day and 5 mg of folic acid by mouth three times a day for the first 10 days of hospitalization

Patients who received vitamin B_{12} and folic acid regained normal appetite more rapidly than those given routine treatment Average duration of illness in the experimental group was 47 5 days compared to 57 2 days for the control group

U S Army Hospital
Kyoto Jap n

ANIMAL STUDIES

135 Shils M E and Stewart W B Development of portal fatty liver in rats on corn diets response to lipotropic agents Proc Soc Exper Biol & Med 85 298 303 Feb 1954

Authors summary Rats subsisting on a diet containing 76% corn and 3% or less casein developed a fatty liver characterized by initial and preponderant accumulation of lipid in the portal areas Rats of the same strain on a diet in which the only protein was casein developed a centrolobular type of fatty liver Methionine choline and vitamin B_{12} were able to decrease the liver lipid However methio-

nine was rarely capable of preventing the deposition of some excess fat while under certain conditions the lipotropic action of vitamin B_{12} was unpredictable

Columbia University College of Physicians and Surgeons New York N Y

136 Bennett, M A, Hellerman, J and Donnelly, A J Liver lesions due to prolonged feeding of a "labile methyl" free diet and the protective influence of vitamin B_{12} Proc Am A Cancer Research 1 4-5, April 1954

The authors anticipated that prolonged feeding of rats on a labile methyl and vitamin B_{12} free diet might produce neoplastic changes in the liver similar to those in rats fed a choline-deficient diet (Am J Path 22 1059 1946)

Twenty four female Lankenau Wistar rats were fed a cystine-free diet of 30 per cent corn oil and 17 per cent amino acids with (group 1) or without (group 2) vitamin B_{12} Other B vitamins were fed daily Group 1 maintained normal liver lipids, an average of 0 15 mcg vitamin B_{12} per Gm dry liver and high body fat Group 2 showed high liver lipids low vitamin B_{12} (average 0 03 mcg per Gm dry liver) and low body fat

Without vitamin B_{12} some damage occasionally severe, was present as early as one year after start of experiment with vitamin B_{12} morphologic evidence of liver disease appeared only in rats on the diet for over 2 years and these lesions were minimal Livers of rats on the vitamin B_{12} free diet showed focal necrosis fatty metamorphosis cirrhosis, duct hyperplasia or cysts Distortions of hepatic architecture in several animals simulated changes which often accompany neoplasms but no lesion could be unequivocally classified as a tumor

Lankenau Hospital Research Institute and Institute for Cancer Research Philadelphia Pa

137 Schweigert, B S Scheid H E and Downing M Liver changes in vitamin B_{12} and riboflavin-deficient rats before and after partial hepatectomy, Am J Physiol 178 338 340, Aug 1954

To determine the role of vitamin B_{12} in the synthesis of nucleic acid rats deficient in vitamin B_{12} and those given supplemental vitamin B_{12} were subjected to partial hepatectomy Comparable studies with rats deficient in riboflavin and those receiving supplementary riboflavin were also conducted

The studies showed that the desoxyribonucleic acid and ribonucleic acid per gram of liver weight or liver nitrogen were reduced in vitamin B_{12} deficiency but not in riboflavin deficiency Moreover a higher mortality and more limited regeneration of liver tissue occurred in the vitamin B_{12}-deficient rats than in the vitamin B_{12} supplemented riboflavin deficient or control rats

These investigations give further evidence of the role of vitamin B_{12} in nucleic acid synthesis However the results should be interpreted with caution since different basal rations were used to produce the two vitamin deficiencies

University of Chicago Chicago Ill

138 Rigdon, R H Couch J R Brashear, D and Qureshi, R T Effect of vitamin B_{12} on selenium poisoning in the rat A M A Arch Path 59 66-72 Jan 1955

Authors summary and conclusions "*Rats given a small amount of selenium in their ration for short intervals* 33 and 48 days have a larger amount of vitamin B_{12} in their livers than have the controls *fed the same ration [without selenium]*

"The daily subcutaneous injection of 200 mcg of vitamin B_{12} in adult rats *apparently does not influence the hepatic necrosis that results from the ingestion of* a small amount of sodium selenite over a period of 17 weeks Vitamin B_{12} does influence however the process of hepatic *repair following injury resulting from se*

lenium The vitamin B_{12} content of the liver is decreased in rats with severe hepatic necrosis resulting from selenium intoxication The vitamin B_{12} content of the liver of rats undergoing regeneration following selenium poisoning is low It is suggested that regenerating hepatic cells either utilize more vitamin B_{12} than do normal hepatic cells or that they fail to store vitamin B_{12} in the same manner in which normal hepatic cells do

University of Texas
S hool of Medicine
Galveston Texas

139 Mallov S Effect of chronic ethanol intoxication on liver lipid content of rats Proc. Soc. Exper Biol & Med 88 246-249 Feb 1955

Vitamin B_{12} and choline were given to alcohol intoxicated rats to determine the effect of lipotropic agents on such animals

The intoxicated rats showed increased liver fat even when diet was adequate but large doses of vitamin B_{12} or choline protected them against fatty livers Doses of 2 9 mcg of vitamin B_{12} daily were ineffective but 6 8 mcg daily was effective

It is stated that these results suggest that alcohol per se has an effect on the liver Since long standing fatty liver may lead to fibrosis and cirrhosis the effect of alcohol may contribute to the development of liver lesions in alcoholics

State Uni rsity of New Yo k Upstate Medical Center
Syracuse N Y

STRESS AND TOXIC STATES

140 Hayes M A Water soluble vitamin requirements in surgical convalescence Ann. Surg 140 661-667, Nov 1954

The author emphasizes the importance of adequate nutrition in relation to surgical procedures and to convalescence after operations

Many patients undergoing major surgical procedures particularly gastrointestinal operations have been nutritionally depleted for a considerable period Inadequate feeding following operation aggravates the effect of loss of vital nutrients

Case histories include records of metabolic studies both before and after intensive vitamin therapy (ascorbic acid thiamine riboflavin, niacinamide pyridoxine calcium pantothenate vitamin B_{12} folic acid and vitamin K) As a result of such therapy carbohydrate metabolism returned to normal hepatic function and hemoglobin synthesis improved and nitrogen balance became positive

Yale Uni e sity
School of Medi i e
New H en Conn

141 Sebrell W H Jr Adequate therapeutic diets J Am Dietet A 30 1256-1259 Dec 1954 (in Current Comment)

Adequate therapeutic diets are discussed with relation to the patient s need for calories protein vitamins and minerals

In the treatment of illness and trauma the vitamins play an important part Thiamine and niacin are useful in carbohydrate metabolism while riboflavin pantothenic acid pyridoxine folic acid and vitamin B_{12} participate in protein and energy metabolism as well as in other processes Ascorbic acid also plays a prominent part in therapeutic nutrition

Vitamin B_{12} is beneficial in very large doses in cranial nerve neuralgias and in diabetic neuritis Spectacular results with vitamin B_{12} therapy in neurologic disorders of alcoholism have recently been described In animal studies preliminary findings indicate that vitamin B_{12} may also promote wound healing

National Institutes of He lth
Bethesda Md

142 Ershoff, B H Decreased resistance of vitamin B_{12} deficient rats to cold stress, Proc Soc Exper Biol & Med 84 615-617, Dec 1953

Author s summary ' 1 Prolonged exposure to cold significantly increased the vitamin B_{12} requirement of the rat 2 Resistance to cold stress as measured by length of survival under conditions of low environmental temperature was significantly impaired in the vitamin B_{12}-deficient rat

Emory W Thurston Laboratories
Los Angeles Calif

143 Kleinsorge, H , Morigerowski, E and Rosner K Anemia in lead poisoning and vitamin B_{12}, Ztschr ges inn Med 9 903, Sept 15 1954 (abstr A M A Arch Indust Health 12 452 453, Oct 1955)

In rabbits rendered anemic by injections of aqueous lead acetate solution administration of vitamin B_{12} with or immediately after the lead dosage resulted in a much more rapid recovery from the anemia Stimulation of the regenerative power of the bone marrow was the cause of the more rapid recovery Vitamin B_{12} also prevented deterioration of the general condition of the rabbits When lead was given with vitamin B_{12} basophilic stippling of the erythrocytes was prevented The authors feel that the vitamin B_{12} either stimulates normal maturation preventing liberation of immature stippled cells or that because it is important in nucleic acid metabolism, it normalizes a pathologic process of ripening which may result from inhibition of the nucleic acid metabolism by lead

144 Hara M Experimental study on the treatment of wounds in cases of hypoproteinosis Nagoya J M Sc 16 12 1953 (Internat Abstr Surg 99 520 1954, in Surg Gynec & Obst , Nov 1954)

The rate and character of healing of simple skin scratches linear incisions and circular skin defects from 5 to 10 mm in diameter were observed in a total of 262 normal and hypoproteinemic mice Hypoproteinemic mice showed poor healing The wound reaction was localized and limited and fibroblasts appeared as late as five days after wounding normal mice showed good fibroplasia by the second and third days Regeneration of blood vessels was also delayed in the wounds of hypoproteinemic mice The only amino acids which had beneficial effects when given singly were methionine, lysine and tryptophan, in the order listed When amino acids with vitamin B_{12} and folic acid were added to the diets of hypoproteinemic mice wound healing approached normal

145 Mecray, P Jr Nutrition and wound healing Am J Clin Nutrition 3 461 465, Nov Dec 1955

The effect of protein and vitamin deficiencies on wound healing is reviewed The fat soluble vitamins presumably play a role in the healing of bone wounds, and the B-complex vitamins are essential to wound healing acting as coenzymes Deficiency of pyridoxine and riboflavin delayed wound healing in rats Vitamin B_{12} increased the strength of rat wounds in the early stages of healing

Cooper Hospital
Camden N J

146 Bloodworth, J M B Jr and Hamwi G J The effect of diet antibiotics and vitamin B_{12} on experimental glomerular lesions produced by cortisone J Clin Endocrinol & Metab 16 944 July 1956 (in Soc Proc)

Previous research showed that administration of cortisone acetate to rabbits caused glomerular lesions resembling those in human beings with diabetes mellitus The authors continued their research by giving to rabbits feeds containing crude antibiotic vitamin B_{12} supplement ' The results were fewer lesions and fewer animals showing lesions Other drug regimens did not produce this effect

Ohio State Uni ersity College of Medicine
Columbus Ohio

147 Bereston E S Vitamins in dermatology Am J Clin Nutrition 2 133 139 March April 1954

The author discusses the use of vitamins in cutaneous diseases Vitamin B_{12} in his experience relieves the pain of herpes zoster good results are also reported in the treatment of seborrheic dermatitis and chronic discoid lupus erythematosus Transient but marked improvement occurs in subacute disseminated lupus erythematosus

University of Ma yla d School of Medicine Baltimore Md

148 Musman D J Psoriasis seborrheic dermatitis and pityriasis rosea Post grad Med 17 85 92 Jan 1955

The causes and treatment of psoriasis seborrheic dermatitis and pityriasis rosea are discussed

After reviewing the results of local application of ointments and of systemic therapy including cortisone and corticotropin the author suggests vitamin B complex liver riboflavin, and vitamin B_{12} as valuable adjuncts in the treatment of these skin diseases

Uni ersity of Colo do School of Medicine Denver Colo

149 Barefoot S W A method of treating chronic leg ulcers North Carolina M J 16 101 102 March 1955

Twenty three patients with chronic stasis ulcers of the leg secondary to varicosities or to old thrombophlebitis were treated locally with antibiotic powder and absorbable gelatin and given vitamin B_{12} parenterally 30 to 45 mcg once a week All ulcers healed satisfactorily within 60 to 75 per cent of the time required when absorbable gelatin and vitamin B_{12} were not used

An ulcer of the nose resulting from severe radiodermatitis healed within four months on the regimen of antibiotics gelatin and vitamin B_{12} Conventional therapy for two years had failed to effect a cure

Greensboro N C

150 Goldblatt S On the intravenous administration of cyanocobalamin Am J Clin Nutrition 3 129 131, March April 1955

No adverse reactions were noted in the course of 3 297 intravenous injections of vitamin B_{12} in 150 patients with various skin neurologic or endocrine diseases The dosage ranged from 15 to 3 000 mcg

The patients ages ranged from 4 to 78 years Daily injections of 1 000 mcg were given to a 10-year-old girl for over 270 days without toxic reactions The largest single dosage used (3 000 mcg) was given daily to a patient for a total of 86 days One patient described burning of the tongue headache and postnasal drip 10 minutes after one injection of 1 000 mcg although she had no other reaction to numerous injections given before and afterward

Uni ersity of Cincinnati Medical School Cincinnati Ohio

151 Neber, H Die Therapie verschiedener Hautkrankheiten insbes allergischer Genese mit Vitamin B_{12} [The treatment of various skin diseases particularly those of allergic etiology with vitamin B_{12}] Munchen med Wchnschr 97 1102 1103 Aug 26 1955

Vitamin B_{12} was used in the treatment of 41 patients with diseases of the skin Ampules containing 1 000 mcg of the vitamin presumably for intramuscular injection were used

Of 11 patients with contact dermatitis 9 improved enough to be in contact with the offending allergen without reaction These patients received 6 000 mcg of vitamin B_{12} in 10 to 14 days

Dermatitis medicamentosa caused by

penicillin-streptomycin in 5 patients and by topical iodine in 4, healed after vitamin B_{12} 1,000 mcg, was given for two days followed by 500 mcg for two days Though such healing could have occurred spontaneously, it is significant that 8 of these patients could continue receiving the offending medication Of 8 patients with eczema of endogenous etiology who received injections of 1,000 mcg of vitamin B_{12}, 4 were relieved but had recurrences 4 were free of itching but lesions remained

Five patients had eczema of the legs associated with varicose ulcers which were being treated with a zinc lime dressing They could tolerate the dressings without itching and rash when they were given two injections of 1 000 mcg of vitamin B_{12} and three later injections of 500 mcg Two patients with severe juvenile acne vulgaris received injections of 1,000 mcg of vitamin B_{12} every other day for 10 days followed by five more injections of 500 mcg every other day In both cases the skin cleared and lesions have not returned in six months

Rosacea cleared in 4 of 5 women who received 500 mcg of vitamin B_{12} every other day for two to three weeks

A man with lichen ruber planus was given two injections of 1,000 mcg of vitamin B_{12} a week for four weeks and then once a week for eight weeks His lesions cleared A recurrence six months later responded to further therapy

The author mentions impressive results obtained by Ruedemann with vitamin B_{12} 1,000 mcg daily in 34 patients with psoriasis

Berlin Charlottenburg Germany

152 Goldblatt, S Lupus erythematosus treated with vitamin B_{12} Acute systemic lupus erythematosus, chronic discoid lupus erythematosus A M A Arch. Dermat & Syph 68 737-739, Dec 1953 (in Soc Proc)

A patient with lupus erythematosus was treated with intramuscular vitamin B_{12} 15 to 120 mcg three times weekly throughout pregnancy She improved with each injection A recurrence of lupus erythematosus was treated with 120 mcg vitamin B_{12} twice weekly Consistent improvement in the general physical condition as well as in the local lesions followed.

Vitamin B_{12} was well tolerated by other patients in doses ranging from 15 to 1 000 mcg for long periods The author states that vitamin B_{12} is a safe, effective agent for the treatment of all types of lupus erythematosus, producing involution of dermal lesions regression of systemic symptoms elevation of pallhypesthesia and correction of anemia

153 Marcus, M D, Conrad A H., Jr and Weiss, R S Treatment of chronic discoid lupus erythematosus with large doses of vitamin B_{12}, J Invest Dermat 21 75 77, Aug 1953

Chronic discoid lupus erythematosus in 17 patients was treated with vitamin B_{12} in intramuscular doses of 1 000 mcg three times a week In 1 patient there was almost complete clearing of the lesions 2 patients were greatly improved 2 slightly improved 9 were unchanged and 3 became worse Most of the patients reported a feeling of well being and increased energy

Barnard Free Skin and Cancer Hospital
St Louis County Hospital and
Washington University School of Medicine
St Louis Mo

154 Brunsting L A Systemic lupus erythematosus South Dakota J Med & Pharm 7 311 313 317, Sept 1954

After evaluating corticotropin and cortisone in the treatment of systemic lupus erythematosus the author states that vitamin B_{12} administered intramuscularly in doses of 15 mg [sic] twice a week for 10 or 12 weeks effects improvement

Mayo Clinic
Rochester Minn

155 Robinson R C V Treatment of xanthelasma and vitamin B_{12} J Invest Dermat 24 111 113 Feb 1955

Vitamin B_{12} was given subcutaneously to 35 patients and orally to 30 with xanthelasma palpebrarum The subcutaneous dosage ranged from 30 mcg to 1 000 mcg weekly for 6 to 20 weeks The oral dose was 1 000 mcg daily for 30 days A favorable result was obtained in 31 of the 35 patients receiving vitamin B_{12} subcutaneously but in only 5 receiving it orally

The process by which vitamin B_{12} ameliorates xanthelasma is apparently not related to its effect on cholesterol metabolism since cholesterol levels measured before and after subcutaneous or oral vitamin B_{12} in 65 patients showed no significant change The reason why oral vitamin B_{12} was ineffective is not clear since blood level measurements showed that it is absorbed from the gastrointestinal tract

Baltimore City Hospitals
Baltimore Md

156 Nicolini A Treatment of erythroderma desquamativum with vitamin B_{12} Minerva pediat 8 53 57 Jan 28 1956 (abstr Med Monatsspiegel 5 136 June 1956)

In 14 cases of desquamative erythroderma in infants an average daily dose of 50 mcg of vitamin B_{12} orally led to striking improvement

Milan Italy

157 Ruedemann R Jr Treatment of psoriasis with large doses of vitamin B_{12} 1,100 micrograms per cubic centimeter A M A Arch Dermat & Syph 69 738 739 June 1954 (in Clinical Notes New Instruments and Techniques)

The author reports on 34 patients with psoriasis who had been treated with intramuscular vitamin B_{12} 1 000 mcg daily for 10 to 20 days followed by a maintenance dose The eruption subsided in 11 patients in 10 it was 75 to 80 per cent improved 6 patients are improving slowly 2 uncooperative patients were not benefited 5 patients had slight recurrences but treatment was resumed with good results Pruritus was relieved in 9 of these patients

The author states that this treatment has surpassed all previous methods in rapid effectiveness lack of reactions and simplicity The rapid response of patients with long standing recalcitrant psoriasis is too consistent to attribute to spontaneous involution Wholesale clinical trials with vitamin B_{12} therefore are indicated

Albany Medical College
Albany N Y

158 Rimbaud P Ravoire J and Rioux J Le traitement du psoriasis par la vitamine B_{12} a 1 000 gammas [Treatment of psoriasis with vitamin B_{12} in a dose of 1000 mcg] Presse méd 64 194 Feb 1, 1956 (in Soc Proc)

For treatment of psoriasis injection of 1 000 mcg of vitamin B_{12} daily for an average of 15 days has been proposed When the authors used it in 17 cases of resistant psoriasis 9 showed sustained improvement 3 had some improvement and 5 none Best results were obtained when there was much congestion and irritation In subseborrheic forms the response was not as good

159 Stiegler, M Traitement du psoriasis par la vitamine B_{12} [Treatment of psoriasis with vitamin B_{12}] Presse méd 64 1719 Oct 20 1956 (in Soc Proc)

Psoriasis in 30 patients was treated with vitamin B_{12} The results were as follows recovery in 3 cases definite improvement in 10 slight improvement in 8 and complete failure in 9 cases

Strasbourg France

NUTRITION AND GROWTH IN CHILDREN

160 Kofman, I Acción estimulante general de la vitamina B_{12} en pediatría [General stimulating effect of vitamin B_{12} in pediatrics], Semana méd 103 261-266, Aug 20 1953

Vitamin B_{12} was given daily to 23 children, all but 2 of whom were under five years of age All were underweight and ate poorly, but were free of overt disease Appropriate changes in diet and administration of calcium salts liver extracts vitamins tonics ' and ultraviolet therapy had already been instituted when indicated before vitamin B_{12} was given

Daily intramuscular injections of 30 mcg of vitamin B_{12} were given to 19 children, and 4 received the same daily dosage orally Total dosage was 300 to 1 850 mcg in 10 to 60 days with an average of 660 mcg in 32 days Increased appetite was observed in 16 children and a significant weight increase in 8 There were no unfavorable side effects

The authors emphasize that although the cause of anorexia should be determined so that the best treatment may be given the empirical use of vitamin B_{12} for anorexia may nevertheless be justified

161 Masi, A and Mori, S Therapeutic activity of liver extracts and vitamin B_{12} in children Minerva pediat 5 290 April 30 1953 (abstr J A M A 153 1129 Nov 21 1953)

Liver extracts combined with vitamin B_{12} were used to treat 30 children with acute respiratory and cardiovascular disease which tended to become chronic despite antibiotic anti infectious and dietetic therapies Atrophy denutrition, asthenia anorexia and pallor were present in all these children The 15 infants from 3 to 15 months old were given 1 Gm of liver extract and 15 mcg of vitamin B_{12} orally every day for 20 to 25 days The infants gained appetite and weight Their general condition improved, and the number of erythrocytes increased as did levels of globulin and protein Only a few under six months of age did not benefit The 15 children from 2 to 15 years old received 2 Gm of liver extract and 15 mcg of vitamin B_{12} intramuscularly every other day for 20 to 25 days Changes in the blood were similar but superior to those in the younger group Oral administration of vitamin B_{12} alone produces only an increase of appetite and of weight when it is combined with liver extracts there is also a slow but progressive improvement of the blood picture

162 Lato M and Biscatti, O Vitamin B_{12} in chronic nutritional disturbances Lattante 23 484 Aug 1952 (abstr A M A Am J Dis Child 86 215, Aug 1953)

Ten dystrophic infants were given 10 mcg of vitamin B_{12} intramuscularly daily for 20 days All gained weight and had an increase in the number of reticulocytes Dystrophy is believed to be due to a conditioned or dietary deficiency of the B vitamins especially of vitamin B_{12}

163 DeMezei M. B The effect of vitamin B_{12} in malnourished infants, Arch argent de pediat 37 31, 1952 (abstr A M A Am J Dis Child 86 674 Nov 1953)

A brief review of the pertinent literature in addition to observations in 7 controls and 8 patients leads the author to conclude that vitamin B_{12} appears to have beneficial effects on weight gain in malnourished infants

164 Karlin R. Variations du taux de vitamine B_{12} dans le lait de femme Effet de l administration de B_{12} sur ce taux [Variations in the level of vitamin B_{12} in mother s milk Effect of the administration of vitamin B_{12} on these levels] Presse méd 63 1608 Nov 19 1955 (in Soc Proc)

Mother s milk is relatively poor in vitamin B_{12} Only colostrum contains an amount approaching that of cow s milk The quantity of vitamin B_{12} diminishes considerably toward the fourth or fifth month of lactation (to 15 to 20 micromicrograms per 100 cc) and drops rapidly toward the end in some cases disappearing completely The enrichment of maternal milk by oral or parenteral administration of vitamin B_{12} to the mother is of interest in the treatment of disorders of growth in infants particularly in debilitated and premature infants Ingestion of the vitamin alone gives variable and generally poor results but its ingestion with intrinsic factor permits a marked augmentation of vitamin B_{12} levels in the milk Intramuscular injection seems to be preferable however to oral administration it produces rapid and marked increases in the vitamin B_{12} content of the milk lasting for 2 to 3 days

165 Kofman I La vitamina B_{12} en altas dosis en la inapetencia infantil [Massive doses of vitamin B_{12} for poor appetite in childhood] Orientación méd 2 1204-1210 Aug 27 1954

Of 674 children brought to the author because their mothers believed they had poor appetites only 10 had poor appetites for no discernible reason. Of these 10 9 had better appetites after receiving 5 to 10 doses of 1 000 mcg vitamin B_{12} either intramuscularly or orally for 10 to 18 days No undesirable side effects were encountered The author emphasized that vitamin B_{12} should not be given indiscriminately for anorexia as there is usually a real cause for poor appetite which will respond to proper treatment

166 Gouyen J and Bouvier R Trial of a product combined vitamin B_{12} and penicillin Semaine d hôp Paris 31 975 977 March 14 1955 (abstr J A M A 158 612, June 18 1955)

Children from 2 months to 8 years of age suffering from weakness hypotrophy and anorexia or convalescing from illnesses were given a tablet containing 200 mcg of vitamin B_{12} and 200 000 units of penicillin No other therapy was given during the trial and no changes were made in the subjects usual diet The treatment was never given during an illness or immediately after an infection Dosage generally ranged from a half tablet to two tablets daily There were 18 weak hypotrophic anorectic children 2 who were convalescing from measles 3 from whooping cough 2 from infectious stomatitis and 5 from various other conditions In evaluating the results of treatment the patient s weight curve appetite and appearance were considered All but one child tolerated the product perfectly and two thirds were unquestionably improved in weight appetite and general condition The rapidity of these gains in the convalescent group further indicated the value of vitamin B_{12} and penicillin

Paris France

167 Wokes F Nutrition and vitamin B_{12} Lancet 2 1343 Dec 24 1955 (Letter to Editor)

Study of data on vegan infants and children indicates that vitamin B_{12} requirements tend to become more critical after early weaning Under such circumstances the use of vitamin B_{12} dietary supplements would seem to be a wise precaution Letters from vegan parents whose children experienced vitamin B_{12} deficiency fully justify this warning

Ovaltine Research Laboratories
King s Langley England

168 Larcomb, J W, Perry, C S and Peterman R A Dietary supplementation of vitamin B_{12} in prepuberty school age children I Growth studies J Pediat 45 70-74, July 1954

In a study of school age prepuberal children on a controlled institution diet 60 received a vitamin B_{12} supplement of 20 mcg daily, 72 received a placebo and all 132 received a daily multiple vitamin supplement containing 1 mcg of vitamin B_{12} activity

The results of this study confirmed earlier reports in that underweight children receiving vitamin B_{12} showed statistically significant weight gains There was no change in the normal children but obese children showed a very slight weight gain No increase in height was seen in underweight or normal children but overweight children showed statistically significant height gain This height gain may suggest that vitamin B_{12} participates in some fundamental metabolic growth regulating mechanism Data on nutritional signs have not yet been analyzed There was no outstanding beneficial effect in the cases of nerve deafness or optic nerve involvement

Ohio State University
College of Medicine
Columbus Ohio
536 Lake Shore Drive
Chicago Ill

169 Patrick, S J Some observations on the metabolism of vitamin B_{12} by Jamaican children, J Nutrition 55 129-135, Jan 1955

A group of 19 Jamaican children on a low intake of animal protein received dietary vitamin B_{12} supplementation 100 mcg daily for nine months Comparison of their plasma concentrations of vitamin B_{12} during the last month of supplementation with those of a control group showed that the supplemented group had significantly higher plasma concentrations

Plasma levels and urinary excretion of vitamin B_{12} after a test dose of the vitamin were determined in 5 children with retarded growth before and after dietary vitamin B_{12} supplementation for nine months, and compared with the values obtained in 5 children with normal growth The latter showed higher plasma levels after the test dose than did the group with retarded growth but the differences were of doubtful significance The test dose caused consistently higher plasma levels in the retarded children after they had received the vitamin B_{12} dietary supplementation No marked differences were observed between the groups in the percentage of the test dose excreted in the urine or in the rate of removal of the vitamin from the plasma

University College of the West Indies
Jamaica British West Indies

170 Campbell J A and McLaughlan J M Vitamin B_{12} and the growth of children a review, Canad M A J 72 259 263, Feb 15, 1955

Authors summary Nine reports on the oral administration of vitamin B_{12} to normal and underweight children and several reports on its use for chronically ill children have been reviewed The reports are conflicting and demonstrate above all, a need for critically controlled experiments before any definite conclusions can be reached regarding the possible effect of vitamin B_{12} on growth in children To date available evidence indicates that the effect is far from spectacular and that there is little if any justification for the wide-spread use of vitamin B_{12}

Food and Drug Laboratories
National Health and Welfare
Ottawa Canada

171 Montoye H J Spata P J, Pinckney V and Barron L Effects of vitamin B_{12} supplementation on physical fitness and growth of young boys J Applied Physiol 7 589 592, May 1955

This study was done as a preliminary step to determine whether supplementary vitamin B_{12} in the diet helps to delay the onset of fatigue after strenuous exercise

The effect of adding 20 per cent protein to the basal diet in the absence of vitamin B_{12} was similar to but less consistent than the effect observed with the high fat diets

National Institutes of Health
Bethesda Md

181 Richardson, L. R Effect of large doses of vitamin B_{12} on reproduction in rats, Federation Proc 13 475, March 1954

"The effect of large quantities of vitamin B_{12} on reproduction in rats was investigated with a synthetic diet which contained soybean protein (Drackett No 220) Two groups of 10 females each received 10 and 25 mcg, and two groups of 20 females each received 0 and 500 mcg of vitamin B_{12}/kg of diet, respectively Another group of 10 females received 100 mcg. of vitamin B_{12}/rat/wk by subcutaneous injection A female was given an opportunity to bear 4 litters and the number of young per litter was reduced to 8 at birth Only 58 per cent of the young observed in 73 litters which were born to females that received no vitamin B_{12} were weaned while 88 1 to 96 1 per cent of young were weaned by those which received vitamin B_{12} There was no evidence that more than 10 mcg was required or that larger quantities were toxic The females receiving vitamin B_{12} by injection produced 39 litters and weaned 87 3 per cent of their young A sixth group of 8 females which had produced 4 litters while receiving vitamin B_{12} at a level of 500 mcg were given 1000 mcg and allowed to bear additional litters Eighty five out of 86, or 98 8 per cent of the young were weaned These data show that large doses of vitamin B_{12} are not injurious to reproduction"

Texas Agricultural Experimental Station
College Station, Texas

182 Wang, H., Scheid, H E and Schweigert, B S Histological studies with rats fed diets containing iodinated casein and different levels of vitamin B_{12} Proc Soc Exper Biol. & Med. 85 382-384, March 1954

Feeding of a vitamin B_{12}-deficient diet to rats caused degenerative changes in the thyroid gland, decreased spermatogenetic activity, shrinkage of the seminiferous tubules and degeneration of interstitial tissues of the testes The thyroid changes were only partially counteracted by vitamin B_{12} while the other symptoms subsided completely in response to vitamin B_{12}

American Meat Institute Foundation, and
The University of Chicago
Chicago Ill

183 Fröhlich, A. Relation between vitamin D and vitamin B_{12}, Nature 174 462-463, Sept 4, 1954

Diets containing high vitamin D supplements but no vitamin B_{12} had a growth depressing effect on chicks. This was counteracted by vitamin B_{12} supplements

National Animal Experimental Station
Uppsala, Sweden

184 Dryden, L P, Hartman, A. M. and Cary, C A Influence of vitamin B_{12} upon vaginal patency in the rat, Proc. Soc. Exper Biol & Med 87 195 197, Oct 1954

Authors summary "The onset of sexual maturity in female rats as measured by vaginal patency, was found to be delayed in vitamin B_{12}-deficient animals as compared to vitamin B_{12} supplemented littermates when their mothers were fed a B_{12}-deficient ration during lactation No such differences were observed when the mothers were fed B_{12} during lactation. The nature of the carbohydrate and protein in the ration was found to have an effect on the differences observed

U S Department of Agriculture
Beltsville Md

185 Fatterpaker, P, Marfatia U and Sreenivasan A. Protective action of vitamin B_{12} in the hyperthyroid rat, Nature 176 165, July 23, 1955

Four groups of rats were maintained on the following regimens (1) basal diet

alone (2) basal diet and vitamin B_{12} (3) basal diet and iodinated casein (4) basal diet iodinated casein and vitamin B_{12} The iodinated casein was given to induce hyperthyroidism and vitamin B_{12} was administered to observe its effects on this condition Percentage weight gains in the four groups during three weeks were as follows (1) 30 5±7 4 (2) 28 2±7 6 (3) —8 5±2 3 (4) —1 8±3 7 Tests made after sacrifice of the animals showed that in the hyperthyroid animals there was a marked reduction in acetylating ability as well as in tissue glutathione content, which was counteracted by vitamin B_{12} supplementation From these results it is concluded that the primary manifestation of thyrotoxicosis is a deficiency of vitamin B_{12}

University of Bombay
Bombay India

186 Ferguson T M., Rigdon R. H and Couch J R. A pathologic study of vitamin B_{12}-deficient chick embryos, A.M.A. Arch Path 60 393-400 Oct 1955

A pathologic study of 139 embryos obtained from vitamin B_{12} depleted hens showed four main categories of lesions (1) decreased size (retarded growth) (2) edema and hemorrhages (3) necrotic foci in the liver brain and spinal cord and (4) increased fat in parenchymatous tissues.

Texas Agricultural and
Mechanical College
College Station Texas
Uni ersity of Texas Medical Branch
Galveston Texas

187 Jones C C Brown S O Richardson, L R. and Sinclair J G Tissue abnormalities in newborn rats from vitamin B_{12} deficient mothers Proc Soc Exper Biol & Med 90 135 140 Oct 1955

The authors conclude that when female rats are deprived of vitamin B_{12} before mating until the end of gestation their progeny are weak and defective Weight of the animals studied was below normal and development was proportionately arrested The kidneys and heart were incompletely developed The primitive reticular cells of the spleen were transformed into large densely chromatic and pyknotic cells Vascular blocks with passive congestion especially in the liver were observed The origin of the fat which blocked the sinusoids of the liver was not determined

Texas Agricultural and
Mechanical College
College Station Texas
University of Texas Medical Branch
Galveston Texas

188 Whipple, G H Robscheit Robbins F S and Bale W F Red cell stroma protein rich in vitamin B_{12} during active regeneration Anemia studies using radioactive cobalt B_{12} in dogs J Exper Med 102 725 731, Dec. 1955

It appears that vitamin B_{12} is an important factor in the early steps of stroma protein formation in the first few days of the life of the red cell in the dog

University of Rochester
Rochester N Y

189 Richardson L R and Brock R. Studies of reproduction in rats using large doses of vitamin B_{12} and highly purified soybean proteins J Nutrition 58 135 145 Jan 1956

Large doses of vitamin B_{12} fed to mother rats were not toxic to the offspring Moreover, the vitamin improved average weaning weight and increased the percentage of the litters that survived

Texas Ag icultural Experiment Station
College Station Texas

190 Jaffé W G Requirements of rats for vitamin B_{12} during growth reproduction and lactation J Nutrition 59 135 146 May 10 1956

Author s summary A rat colony was kept on a fortified soybean oil meal-corn ration low in vitamin B_{12} for 18 genera

tions using mostly brother and sister matings Litters starting with the second generation showed high mortality, low birth weights, low weaning weights slow post weaning growth and low liver and kidney vitamin B_{12} levels Females of this group were older when giving birth to their first litters than the controls Blood characteristics and liver glutathione levels were normal No significant difference between succeeding generations could be detected and therefore no indication for a genetic selection toward resistance to vitamin B_{12} deficiency could be found

"The addition of 3 mcg of vitamin B_{12} per kilogram of diet eliminated most of the deficiency symptoms but did not result in optimal weaning weights and post weaning growth while supplements of 5 mcg of vitamin B_{12} per kilogram of diet, or 30 mcg of this vitamin together with 0 2% of methionine gave identical results in overcoming these deficiency signs All of the animals used had been kept for at least one generation on the respective experimental diets previous to the experiments presented

Instituto Nacional de Nutrición
Caracas Venezuela

191 Allen, S H The effects of vitamin B_{12} deficiency and of copper deficiency on the concentration of free protoporphyrin in the erythrocytes of sheep, Biochem J 63 461-469, July 1956

In vitamin B_{12}-deficient sheep the concentrations of free protoporphyrin in the red blood cells is invariably much higher than normal it increases more or less steadily as anemia develops

University of Adelaide
Adelaide Australia

192 Ferguson T M Rigdon, R H and Couch J R Thyroid in B_{12} deficient chick embryos Endocrinology 60 13-21 Jan 1957

Authors summary 'The histologic characteristics of the thyroid gland in vitamin B_{12} deficient chick embryos are described The rate of development of the thyroid in the vitamin B_{12} deficient embryos is 24 to 72 hours behind that of the normal chick embryo The gland from the deficient embryo is larger the follicles vary in size, and the amount of colloid is less than that present in the normal embryo This study suggests that the thyroid from vitamin B_{12} deficient embryos may be hypofunctional

It was found that the vitamin B_{12} content of eggs from hens fed an adequate diet ranged from 4 to 15 mcg per Gm of yolk while eggs from vitamin B_{12}-deficient hens contained 0 2 to 4 mcg per Gm of yolk.

Texas Agricultural and
Mechanical College
College Station Tex

University of Texas Medical Branch
Galveston Texas

193 Ferguson, T M, Trunnell J B Dennis, B, Wade, P and Couch J R The influence of vitamin B_{12} deficiency on the uptake of I^{131} by the thyroid gland in adult and embryonic chickens, Endocrinology 60 28 32 Jan. 1957

Authors summary 'The thyroid glands found in embryos from B_{12}-deficient hens exhibit reduced ability to concentrate I^{131} Monoiodotyrosine diiodotyrosine thyroxine and iodide are present in the deficient glands as indicated by paper radio-chromatography

The I^{131} uptake of pullets of hens fed a B_{12}-deficient diet may be somewhat increased by administration of vitamin B_{12}

There is marked reduction bordering upon absence of sulfhydryl groups in the thyroid glands of vitamin B_{12} deficient embryos and hens

Texas Agricultural and
Mechanical College
College Station Texas

University of Texas
Galveston Texas

M D Anderson Hospital and
Tumor Institute
Houston Texas

194 Zarrow M X Horger L M and McCarthy J L Atrophy of adrenal gland following thiouracil and vitamin B_{12} Proc. Soc. Exper Biol & Med 94 348 349 Feb 1957

Thiouracil administration causes growth inhibition and adrenal atrophy in rats In this experiment rats were given vitamin B_{12} 10 or 20 mcg daily intraperitoneally for 12 weeks along with the oral thiouracil Vitamin B_{12} prevented growth inhibition but failed to prevent adrenal atrophy Thus adrenal atrophy following administration of thiouracil is not a reflection of generalized growth inhibition but must be attributable to a specific action of thiouracil on the adrenal

Purdue University
Lafayette Ind

NUTRITION IN THE ELDERLY

195 Chow B F Vitamin B_{12} and aging Federation Proc 13 453-454 March 1954

In studies comparing the absorption and disposition of vitamin B_{12} administered orally and parenterally to 8 young persons (20 to 40 years) and to 18 old subjects (60 to 90 years) the author notes several significant differences

(1) Old persons retain a larger portion of injected vitamin B_{12} than do the young

(2) Samples of gastric juice from old subjects who did not have achlorhydria contained less vitamin B_{12} binding substance than similar samples from the young group

(3) The mean level of vitamin B_{12} serum of the old group was significantly lower than that of the young

(4) Oral tolerance tests showed that all 8 young subjects in comparison with only 6 of the 18 old subjects after receiving 1 0 mg vitamin B_{12} orally responded with a marked elevation in blood level After the test dose was lowered to 250 mcg none of the 10 old subjects showed an increase however 5 of the 8 young persons responded with an elevation of the blood level

These studies indicate that because of lack of intrinsic factor older people are less able to absorb exogenous vitamin B_{12} and thus the vitamin B_{12} level is lowered in blood and tissues

Johns Hopkins University
Baltimore Md

196 Chow B F The absorption of vitamins and enzymic activities in aging Internat Rec Med 167 429-430 July 1954 (in Soc Proc)

A test for determining absorption and utilization of vitamin B_{12} consists of obtaining a fasting blood sample then feeding a large dose of vitamin B_{12} withdrawing of two additional blood samples 1½ and 3 hours later and analyzing all the blood samples for vitamin B_{12} content The normal level of vitamin B_{12} in young individuals was approximately 250 micromicrograms and there was a 90 per cent response to a 1 mg dose of oral vitamin B_{12} in 10 out of 11 young subjects

Of 36 people averaging 70 years of age a 40 per cent average response occurred in 14 after a similar dose Old people have a lowered vitamin B_{12} binding capacity generally reflected by their increased tendency toward achlorhydria perhaps they have a lowered content of intrinsic factor available for binding vitamin B_{12} When vitamin B_{12} is injected in old individuals more of it is retained than in the young This is interpreted to mean that there is less tissue saturation in old subjects hence they retain more of the injected vitamin

Johns Hopkins University
Baltimore Md

197 Boger, W P, Wright, L D, Strickland, S C, Gylfe, J S and Ciminera, J L Vitamin B_{12} correlation of serum concentrations and age, Proc Soc Exper Biol & Med 89 375-378, July 1955

Authors summary ' Total serum vitamin B_{12} concentrations were determined in 528 individuals by the *L leichmanni* method, and a justification for the choice of this method of assay is presented The individuals studied were normal as far as physical health is concerned, and the major variable was age In such persons there is a trend toward lower serum concentration of vitamin B_{12} in the aged than in younger age groups Although this difference is statistically significant, the biological importance of this finding is not apparent There is wide individual difference of values in serum vitamin B_{12} concentration at all age levels but in this normal group values below 200 μmcg /ml and above 1000 μmcg /ml were uncommon The average for the entire 528 individuals was 560 μmcg /ml the 95% confidence bands being 70 and 1060 μmcg /ml respectively

Norristown State Hospital
Norristown, Pa

Sharp & Dohme
Division of Merck & Co Inc
West Point Pa

198 Monat, H A Nutrition of aged cardiac patients, Geriatrics 10 581 582 Dec 1955

The author states that the majority of aged cardiac patients are in a deplorable nutritional state and that the various cardiac drugs reduce the desire for food He recommends a diet allowing 20 calories 1 Gm of protein and 0 5 Gm of fat per Kg of body weight the rest being allotted to carbohydrates Necessary daily dietary adjuncts include calcium iron 25 000 I U of vitamin A, 10 mg of thiamine 10 mg of riboflavin 16 mg of niacin, 5 mcg of vitamin B_{12} 2 mg of folic acid 150 mg of ascorbic acid and 1,000 I U of vitamin D In addition when the patient is first placed on the above regimen, daily intramuscular injections of 30 mcg of vitamin B_{12} and 100 mg of thiamine should be given for 30 days or until definite improvement is evident The author comments Paradoxically this calorically submaintenance diet in a short time improves the patient s general physiologic processes

Washington D C

199 Zintel, H A Nutritional rehabilitation of the elderly patient undergoing surgery, Am J Clin Nutrition 3 501 510 Nov -Dec 1955

The writer believes that all elderly patients need supplementary vitamins especially after surgical operation or protracted illness He states that vitamin B_{12} is a potent antianemic substance and is important in transmethylation, and in the utilization of marginal intakes of protein He states It is well to remember that (1) the clinical avitaminoses are rare (2) the subclinical deficiencies are common especially in the aged (3) the subclinical deficiencies have multiple and profound influences on the physiologic processes of the body and (4) for optimum therapy the physician must assume that all ill elderly patients are deficient

St Luke's Hospital
New York N Y

200 Kaufman W The use of vitamin therapy to reverse certain concomitants of aging, J Am. Geriatrics Soc 3 927 936, Nov 1955

In 35 patients over 45 years of age, none of whom had tropical sprue or pernicious anemia the constant symptoms were mild dysequilibrium, impaired memory and concentration, depression insomnia nervous irritability and fatigue Less constant symptoms consisted of breathlessness with impaired heart sounds dyspepsia paresthesias, and difficulty in controlling the bladder These symptoms

disappeared within two weeks after the patients received intramuscular injections of 100 mcg of vitamin B_{12} once or twice a week After three months of this therapy some patients had recurrences within a week after treatment was discontinued but reinstitution of therapy alleviated the symptoms Most patients receiving this treatment remained well for three to nine months

Bridgeport Conn.

201 **Glass G B J Goldbloom A A, Boyd L J Laughton R. Rosen S and Rich, M Intestinal absorption and hepatic uptake of radioactive vitamin B_{12} in various age groups and the effect of intrinsic factor preparations Am J Clin Nutrition 4 124 133 March April 1956**

Measurement of hepatic uptake of 0 5 mcg of Co^{60} vitamin B_{12} orally administered to 60 individuals from 18 to 90 who were normal or had some irrelevant disorder indicates wide variation in the intestinal absorption of vitamin B_{12}

There is no evidence of general impairment of intestinal absorption of vitamin B_{12} as a function of advancing age but more older than younger individuals have impaired intestinal absorption of vitamin B_{12}

In individuals over 60 absorption is significantly lower in subjects with gastric hypo- or anacidity than in those with normal or hyperacid gastric secretion Measurement of the hepatic uptake of Co^{60} vitamin B_{12} might be helpful in the early recognition of vitamin B_{12} absorption defects of a sprue-like or pernicious anemia like type in this group

Addition of a potent intrinsic factor preparation increases hepatic uptake of radioactive vitamin B_{12} over 25 per cent in almost half the subjects in all age groups Especially important is the increased hepatic uptake in older individuals with decreased gastric acidity which is usually associated with reduction of other gastric secretions

Intrinsic factor preparations appear helpful not only in states of frank vitamin B_{12} deficiency such as pernicious anemia or total gastrectomy but also in clinically latent partial defects of absorption

New York Medical College
Flower and Fifth Aven e Hospitals and
Bird S Coler Memorial Hospital and Home
New York N Y

202 **Chow B F, Wood R Horonick A and Okuda K. Agewise variation of vitamin B_{12} serum levels J Gerontol 11 142 146 April 1956**

Serum vitamin B_{12} levels were measured in young and old individuals and in rats by means of the microbiologic method of Skeggs and Wright Preliminary studies revealed that vitamin B_{12} serum levels were not influenced by dietary intake immediately preceding the drawing of blood samples and were relatively constant in the same individuals measured at different times

Study A There were 210 subjects 65 young (age 25 to 35) students technical assistants or prisoners and 145 old individuals (prisoners inmates of homes for the aged and members of a club who lived at home) The young subjects had definitely higher serum vitamin B_{12} levels than the old ones For example the mean vitamin B_{12} value in mcg per cc of serum for young students and technicians was $231\ 5 \pm 27\ 6$ while that of a group in an old age home was $151\ 8 \pm 18\ 7$

Study B In this group there were 114 healthy subjects ranging in age from 10 to over 70 selected at random from the Baltimore population Again the vitamin B_{12} content of serum definitely decreased with advancing age

Study C The findings in human beings were confirmed in rats Both male and female rats showed statistically significant decreases in serum vitamin B_{12} levels with advancing age

The authors conclude that the advisability of administering vitamin B_{12} to certain old individuals in order to bring

the serum level of vitamin B_{12} to normal needs further study since the clinical effect of this measure has not been determined

Johns Hopkins University
Baltimore Md

Geriatric Institute of the
Daughters of Jacob
Bronx N Y

203 Blumberg N and Zisserman L Diabetes in the older age group Geriatrics 11 444-446, Oct 1956

In an article devoted primarily to diabetes in the aged the authors refer to studies now in progress aimed at revealing physiologic aspects of vitamin B_{12} in the aged It is stated that there is a relatively low serum level of vitamin B_{12} in aged individuals as a group and that glucose tolerance seems to be directly related to the vitamin B_{12} serum level The exact significance of these findings is not yet clear

Home for the Jewish Aged
Philadelphia Pa

204 Gaffney G W, Horonick, A Okuda K, Meier P Chow, B F and Shock, N W Vitamin B_{12} serum concentrations in 528 apparently healthy human subjects of ages 12 to 94, J Gerontol 12 32-38 Jan 1957

Serum vitamin B_{12} levels were determined in healthy subjects ranging in age from 12 to 94 Of the 528 subjects 89 were in patients of a gerontology branch of Baltimore City Hospital 55 were physicians or staff members of the hospital ranging in age from 20 to 49, 97 were male prison inmates receiving an adequate diet 161 were individuals from the Baltimore population and 126 were healthy residents or staff members of a private home for the aged

Blood was withdrawn in the morning from fasting patients and its vitamin B_{12} content was assayed by a microbiologic method An almost uniform gradual decline in mean serum vitamin B_{12} levels was noted with advancing age in all groups Low serum vitamin B_{12} levels were found more frequently in persons over 50 years old than in younger persons There was no substantial difference in serum vitamin B_{12} levels of men and women

The authors conclude Available evidence supports the contention that observed regression of serum B_{12} levels on age is statistically significant The agreement in regression lines between the several groups widely different in background and living conditions, suggests that the B_{12} level is relatively unaffected by these conditions If dietary conditions per se play a significant role in the agewise decrease in serum B_{12} in the groups studied they do so in consequence of an age dependent alteration in the selection of foods by the subjects themselves

National Institutes of Health
Bethesda Md

Johns Hopkins University and
Baltimore City Hospitals
Baltimore Md

Geriatric Institute of the
Daughters of Jacob
Bronx N Y

205 Tauber S A, Goodhart R S, Hsu J M, Blumberg, N, Kassab, J and Chow, B F Vitamin B_{12} deficiency in the aged, Geriatrics 12 368 374, June 1957

Serum levels of vitamin B_{12} and the glutathione content of erythrocytes were determined in 20 young and 21 old healthy individuals The serum vitamin B_{12} levels of the old people were significantly lower than those of the young people 160±14 5 and 297±17 7 micromicrograms per cc respectively Likewise the glutathione content of the erythrocytes in the old individuals was significantly lower than in the young people It has been shown that erythrocyte glutathione levels are low in vitamin B_{12} deficiency in rats and man These findings thus provide additional evidence of the existence of a state of vitamin B_{12} undernutrition in the aged

the mother In 89 of 96 (92 per cent) of the cases the infant s vitamin B_{12} concentration was higher than that of the mother the differences being in some cases as much as sixfold in favor of the infant A large number, 28 of 96 (29 per cent) of the umbilical-cord serum values were above 1060 micromicrogm per milliliter, which has been defined as the upper band of 95 per cent confidence limits for 'normal adults This seeming avidity of the infant for vitamin B_{12} has not been explained

The lowered serum vitamin B_{12} concentration due to pregnancy was confirmed Occasional unexplained high values for serum vitamin B_{12} were again observed (vitamin administration was eliminated as a possible cause)

' The importance of determining normal values concurrently in the same institution from which abnormal groups are derived is emphasized

Norristown State Hospital
Norristown Pa

210 **Baker, H, Erdberg R, Pasher, I and Sobotka H Study of folic acid and vitamin B_{12} in blood and urine during normal pregnancy, Proc Soc Exper Biol & Med 94 513 515, March 1957**

Blood and urine levels of vitamin B_{12} and folic acid were determined in 47 women during pregnancy Vitamin preparations free of vitamin B_{12} and folic acid were given but the women were not on a controlled diet Assays of vitamin B_{12} used *Euglena gracilis* and *Ochromonas malhamensis* assays of folic acid used a thermophilic bacillus

The median urinary vitamin B_{12} level was 55 micromicrograms per cc the median blood level was 193 micromicrograms per cc Moreover the majority of the urinary levels were below 60 micromicrograms per cc and one third of the blood levels were below 125 micromicrograms per cc These median values are two to five times lower than normal for urine and four times lower for blood The low serum vitamin B_{12} levels observed as pregnancy progressed suggest that the fetus may attract maternal vitamin B_{12} across the placenta, thereby depleting this vitamin in the maternal circulation

The median level of folic acid in urine and blood was 19 micromicrograms per cc, two to eight times as high as normal The authors state that high maternal stores of vitamin B_{12} and folic acid during pregnancy should alleviate some of the stress which fetal development places upon maternal metabolism and could prevent intrauterine injury

Mt Sinai Hospital
New York N Y

211 **Hellegers A, Okuda, K, Nesbitt, R. E L, Jr, Smith D W and Chow, B F Vitamin B_{12} absorption in pregnancy and in the newborn, Am J Clin Nutrition 5 327 331 May June 1957**

Absorption of vitamin B_{12} was measured in pregnant and nonpregnant women and rats Thyroid activity and serum vitamin B_{12} levels were studied in 2 children with cretinism

Oral doses of 1 000 mcg 500 mcg and 250 mcg were given to pregnant and nonpregnant women and the serum vitamin B_{12} levels were measured before administration and 90 minutes and three hours afterward A positive response (increase of 160 mcg or more) occurred in 8 of 9 pregnant subjects but in only in 2 of 7 nonpregnant women at the higher dose Differences between the two groups using the lower dosages were not statistically significant

Serum vitamin B_{12} levels were measured in normal rats before mating and two days before delivery The vitamin B_{12} serum level decreased to about half its original value during pregnancy Absorption was also studied in rats before mating and near delivery and in nonpregnant

controls A dose of 50 micromicrograms of radioactive vitamin B_{12} was given orally followed by injection of the same amount of unlabelled vitamin B_{12} two hours later Radioactivity of the urine feces and fetus was measured The preg nant animals showed about a twofold in crease in absorption and 60 per cent or more of the absorbed vitamin was con centrated in the fetus Co^{60} vitamin B_{12} 10 micromicrograms was injected into pregnant and nonpregnant rats Assay of liver kidney and fetus showed that the target organs of pregnant animals con tained much less radioactivity than those of controls and the absorbed vitamin B_{12} was concentrated in the fetus

The authors have reported (in press) that in spite of low maternal serum vita min B_{12} levels, the fetus could show ade-quate or high serum levels They have seen 2 instances in which neither mother nor fetus showed appreciable serum lev els of vitamin B_{12} and the latter showed cretinism The mother s serum vitamin B_{12} level returned to normal without thyroid or vitamin B_{12} administration, but the infant s serum vitamin B_{12} level did not return to normal until thyroid therapy was given, and then the rise was rapid The authors suggest that the fetal serum vitamin B_{12} level rather than the maternal level is significant in cretinism Further studies on cretin infants showed that the administration of thyroid extract to cretins will rapidly lead to return of the vitamin B_{12} levels to normal

Johns Hopkins University
Baltimore Md

METABOLIC STUDIES IN HUMANS

212 Editorial Vitamin B_{12} research J A M.A. 153 960 Nov 7, 1953

At a meeting of scientists from many fields evidence was presented that indi cates an important role of the vitamin [B_{12}] in the synthesis of nucleic acids and in the formation or function of such metabolically active compounds as glycine serine methionine and choline Interrelationships between vitamin B_{12} and other vitamins such as pyridoxine and pantothenic acid were demonstrated Additional evidence for the interdepen dence of vitamin B_{12} and the folic acid series was presented and the role of intrinsic factor was reviewed Growth studies were reported emphasizing the importance of adequate supplies of the vitamin in the metabolism of carbohy drate and fat, including not only the con version of carbohydrates to fat but the metabolism of fat itself

Vitamin B_{12} has been shown to play an important role in modifying the con centration of sulfhydryl-containing com pounds of physiological importance The hypothesis was presented that in the absence of adequate supplies of the vita min coenzyme A would not be reduced from the disulfide to the sulfhydryl form and would therefore inhibit the normal pathways of fat metabolism

213 Kaye R, Caughey R H and McCrory W W The minimal ni trogen requirement of infants and the effect of vitamin B_{12} upon it A M.A Am J Dis Child 86 326-327 Sept 1953 (in Soc Proc)

Experiments to determine the minimal nitrogen requirement for equilibrium in 5 boy babies showed that, on an average intake of 98 mg of nitrogen per Kg the nitrogen retention averaged —2 mg per Kg per day and the average weight gain was 1 Gm per Kg per day the 2 patients given more than 100 mg per Kg per day were in positive balance Three similar subjects given an average nitrogen intake of 870 mg per Kg per day showed an average nitrogen retention of 269 mg per Kg per day and a weight gain of 13 Gm per Kg per day

Oral doses of 30 to 300 mcg of vita

min B_{12} per day did not increase nitrogen retention in the high protein subjects in fact, the average retention decreased from 269 to 213 mg a day but as this represented only 5 per cent of the intake it was not considered significant In the low protein group vitamin B_{12} increased average nitrogen retention from 3 to 20 mg per Kg per day but this was of questionable significance because most of the increase was accounted for by one infant Changes in rate of weight gain were in the same direction as changes in nitrogen balance

Vitamin B_{12} administration was associated with an increase of 25 per cent in urinary nitrogen excretion animal experiments of others suggest that this might be a reflection of an enhanced conversion of amino acids into fat or carbohydrate

Philadelphia Pa

214 Kaye R, Caughey R H and McCrory W W Effects of vitamin B_{12} and Aureomycin on nitrogen retention in infants Pediatrics 13 462-475, May 1954

The effects of vitamin B_{12} on weight, and on nitrogen and electrolyte balances were studied in 6 male infants Five appeared to have made subnormal growth progress and 1 was recovering from Leiner's disease Three of the infants were offered nitrogen intakes of approximately 1 0 Gm per Kg per day and the remaining 3 an intake of 0 1 Gm per Kg per day

The authors were unable to obtain unequivocal evidence that vitamin B_{12} exerted a nitrogen anabolic effect in these infants Those on a high protein intake had an increased urinary nitrogen excretion which may have reflected an enhanced rate of conversion of protein into carbohydrate or fat Averages of the control and vitamin B_{12} period nitrogen retentions and weight gains in Gm per Kg per day were 0.235 and 11 8 for those fed high protein diets and 011 and 2 4 for those fed low protein diets

Appetite stimulation was noted in 2 of the 6 subjects given B_{12}

Children s Hospital of Philadelphia and University of Pennsylvania School of Medicine Philadelphia Pa

215 Wokes F and Picard C W The role of vitamin B_{12} in human nutrition, Am J Clin Nutrition 3 383-390 Sept-Oct 1955

Hypotheses of the role of vitamin B_{12} in metabolic systems are formulated The theories involve the assumption that hydroxocobalamin (or aquocobalamin) rather than cyanocobalamin is the active form of vitamin B_{12} The presence of cobalt in an organic coordination complex permits cyanide to be taken up by the vitamin The authors present a hypothetical scheme of cyanide utilization which suggests a mechanism for increased thiocyanate excretion in deficient subjects and offers an explanation of the methionine or choline sparing action of vitamin B_{12} Either methionine or cysteine could act as sulfur donors in this system A hypothetical sulfur cycle is outlined which the authors believe occurs in the liver

Ovaltine Research Laborat ries King s Langley England

METABOLIC STUDIES IN ANIMALS

216 Doctor, V M Welch B E, Perrett R W Brown C L, Gabay, S and Couch J R Metabolic interrelationship between folic acid vitamin B_{12} and the citrovorum factor Proc Soc Exper Biol & Med 84 29 32 Oct 1953

Supplementing a low vitamin B_{12} PGA (pteroylglutamic acid) chick diet either with increasing levels of vitamin B_{12} or increasing levels of PGA resulted in a greater capacity by the liver homogenates to convert added PGA to CF (citrovorum factor)

Texas Agricultu al Expe iment Station College Station Texas

217 Firth, J Mistry S P James M F and Johnson B C. Vitamin B_{12} and transmethylation in the baby pig Proc. Soc. Exper Biol & Med 85 307 309 Feb 1954

The effect of a vitamin B_{12} deficiency on transmethylation from methionine to choline was studied in the baby pig The deficiency did not influence the level of choline in the liver nor the excretion of urinary choline Histologic examination of livers and kidneys did not reveal the fatty infiltration or renal damage characteristic of a choline deficiency This study indicated that vitamin B_{12} is not involved in this direct transmethylation

University of Illinois Urbana, Ill

18 Vitamin B_{12} and methyl group synthesis Nutrition Rev 12 218 220 July 1954

The literature on the influence of vitamin B_{12} on the synthesis of methyl groups is reviewed It appears that vitamin B_{12} reduces the absolute dietary requirements for the methyl group apparently by promoting the biosynthesis of this group

219 Fatterpaker P Marfatia U and Sreenivasan A Role of folic acid and vitamin B_{12} in transmethylations Part I. Formation of creatine in vitro and in vivo Indian J M Research 43 43 50 1955 (abstr Blood 11 685 July 1956)

Deficiency of either folic acid or vitamin B_{12} or both led to a diminution in creatine formation and excretion The effect was especially seen in vitro synthesis in vivo it was less pronounced in the urine and least in skeletal muscle On the administration of large doses of folic acid or vitamin B_{12} to the deficient animals there was rapid improvement in the impaired creatine metabolism In contrast addition of folic acid or vitamin B_{12} did not activate in vitro the creatine forming enzymes in the deficient systems

University of Bombay Bombay India

220 Verly, W G and Cathey W J The influence of vitamin B_{12} on the biosynthesis of the methyl group of choline from methanol J Biol Chem 213 621-624 April 1955

Methyl synthesis from C^{14} methanol in rats deficient in vitamin B_{12} and doubly deficient in vitamin B_{12} and folic acid was compared with that in vitamin B_{12} treated controls The deficient animals incorporated less isotope into the methyl group of choline than the controls Animals deficient in folic acid as well as vitamin B_{12} showed less incorporation than animals deficient in vitamin B_{12} alone

Cornell University Ithaca N Y

221 Boxer, G E Shonk C E Gilfillan E W, Emerson G A and Oginsky E L Changes in coenzyme A concentration during vitamin B_{12} deficiency Arch. Biochem & Biophys 59 24 32 Nov 1955

A striking increase in coenzyme A (CoA) concentration in the liver of vitamin B_{12}-deficient chicks has been preliminarily reported These data needed confirmation in another species Data on the CoA concentration in the liver and other organs of vitamin B_{12}-deficient rats have therefore been obtained and the state of reduction of the increased CoA in liver has been determined

Of the 49 rats used 20 received diets deficient in vitamin B_{12} 29 received vitamin B_{12} supplements and 10 in each of the two groups also received thyroid powder

The CoA concentration in the liver and kidney of vitamin B_{12}-deficient male and female rats is two to three times higher than in controls This increase is independent of the added stress of thyroid powder feeding

The increased CoA is in the catalytically active reduced form The rate of degradation of CoA is the same in homogenates of livers from deficient and normal animals

Merck & Co Inc
Rahway N J

222 Wong, W T and Schweigert, B S Role of vitamin B_{12} in nucleic acid metabolism II Liver coenzyme A levels in the rat, Arch Biochem & Biophys 60 126-129, Jan 1956

The present studies were designed to determine the coenzyme A (CoA) levels in livers of vitamin B_{12}-deficient and supplemented rats to provide further information on the role of this vitamin in metabolism

Some weanling rats from mothers fed diets deficient in vitamin B_{12} and casein were fed similar diets for six weeks, while others received supplements of vitamin B_{12} After sacrifice the CoA content of their livers was determined

The deficient groups showed a definitely smaller weight gain and other abnormalities characterized by a lower content of liver nucleic acids The CoA level was two to three times greater in the livers of vitamin B_{12} deficient animals than in those of the controls No overlapping in the range of values was noted

American Meat Institute Foundation and
University of Chicago
Chicago Ill

223 Wong, W T and Schweigert, B S Role of vitamin B_{12} in nucleic acid metabolism I Hemoglobin and liver nucleic acid levels in the rat J Nutrition 58 231 242 Feb 10 1956

Authors summary 'The growth rate liver DNA [desoxyribonucleic acid] and RNA [ribonucleic acid] levels and liver regeneration ability after partial hepatectomy of rats fed an [0 06%] iodinated casein basal diet deficient in vitamin B_{12} were lower than those of supplemented controls

In subsequent experiments an uncomplicated vitamin B_{12} deficiency was produced in rats born from mothers fed the vitamin B_{12}-deficient diet without iodinated casein during gestation and lactation The growth rate and liver nucleic acid composition of these rats fed the basal ration were significantly lower than those for the controls fed supplemented rations A syndrome characterized by presence of porphyrin whiskers and scaly feet was also noted in the deficient rats

The addition of dried liver to the non iodinated casein ration did not improve the growth promoting activity of vitamin B_{12} when the latter was present at a level of 50 mcg /kg ration However, inclusion of 4 or 10% of dried liver in the diet of the female rats, and 10% of liver in the diet of male rats significantly increased the hemoglobin content of the blood as compared with that observed when the vitamin B_{12}-deficient diet was fed The significance and implications of these findings were discussed

University of Chicago and
American Meat Institute Foundation
Chicago Ill

224 Register, U D Effect of vitamin B_{12} on liver and blood nonprotein sulfhydryl compounds, J Biol Chem 206 705, 1954 (abstr Am J Clin Nutrition 2 373, Sept Oct 1954)

Weanling rats born to mothers maintained on vitamin B_{12} deficient diets were continued on the deficient diets, and their weight gains liver and blood values for ergothioneine, glutathione and total non protein sulfhydryl groups were compared with those in animals receiving the same diet plus injections of vitamin B_{12} There was a marked decrease, largely due to glutathione in the levels of liver and blood sulfhydryl groups in the deficient animals There was no significant effect on the liver or blood ergothioneine

225 Ling C. T and Chow, B F The influence of vitamin B_{12} on carbohydrate and lipide metabolism J Biol Chem. 206 797-805 Feb 1954 (abstr Blood 9 930 Sept 1954)

In vitamin B_{12}-deficient rats glucose tolerance tests produced abnormally high blood sugar levels Such rats consistently showed lower blood sugar levels after short periods of fasting but developed hunger diabetes sooner than control animals The phospholipide content of the body tissues of such deficient rats was much less per unit weight than the phospholipide content in the tissues of vitamin B_{12}-treated rats In two patients with pernicious anemia in relapse the phospholipide content of the blood was low but rose in treatment with B_{12} A high carbohydrate-low fat diet supplemented with injections of glucose solution produced in normal rats not only a hyperglycemia but also concomitant decrease in glutathione content of the blood Administration of glutathione produced transient but significant drops in the blood sugar of these animals As vitamin B_{12}-deficiency tends to lower blood glutathione it is suggested that the maintenance of an adequate concentration of glutathione may be one of the roles of vitamin B_{12} It is suggested also that glutathione may be involved in the utilization of carbohydrate Thus it is concluded that vitamin B_{12} plays an important role in carbohydrate and lipide metabolism and that its effect on blood glutathione concentration may be of significance in its role in metabolism

J hns Hopkins University
School of Hygiene and Public Health
Baltimore Md

226 Ling C T and Chow B F Effect of vitamin B_{12} on ribose formation in erythrocytes Federation Proc 13 253 March 1954

Our previous studies indicate that (1) vitamin B_{12}-deficiency results in impaired carbohydrate metabolism and decreased blood glutathione concentration and (2) an interrelationship exists between glucose utilization and glutathione concentration Several investigators have presented evidence implicating vitamin B_{12} in the synthesis of nucleosides or nucleic acids It has been found that whereas thymidine and other desoxyribosides or desoxyribonucleic acid may replace vitamin B_{12} for the growth of certain microorganisms thymine cannot Since thymine differs from thymidine in that it lacks desoxyribose vitamin B_{12} may be concerned with the sugar moiety of the nucleosides or nucleic acids The influence of this vitamin on carbohydrate metabolism and nucleic acid synthesis could be readily comprehensible on this basis In this connection, it should be interesting to ascertain whether vitamin B_{12} influences the formation of ribose in tissue cells The first phase of this study concerns the *in vitro* formation of ribose in erythrocytes with glucose as substrate Incubation of erythrocytes in isotonic phosphate buffer containing glucose under aerobic conditions for 2 to 3 hours resulted in a marked increase of ribose in the erythrocytes These results suggest that ribose formation may be an important pathway of glucose utilization in mammalian erythrocytes Red cells from B_{12}-deficient rats showed significant depression of ribose formation and oxygen consumption as compared with erythrocytes from B_{12} repleted or normal animals Addition of reduced glutathione to the incubation mixture restored the normal rate of ribose synthesis and oxygen consumption

Jefferson Medical College
Philadelphia Pa

Johns Hopkins School of Hygie e and Public Health
Baltimo e Md

227 Meites J and Feng Y S L. Counteraction by vitamin B_{12} of protein catabolic effects of cortisone Federation Proc 13 468-469 March 1954

Some investigations have indicated that large doses of vitamin B_{12} partially

The increased CoA is in the catalytically active reduced form The rate of degradation of CoA is the same in homogenates of livers from deficient and normal animals

Merck & Co Inc
Rahway N J

222 Wong W T and Schweigert, B S Role of vitamin B_{12} in nucleic acid metabolism II Liver coenzyme A levels in the rat, Arch Biochem & Biophys 60 126-129, Jan 1956

The present studies were designed to determine the coenzyme A (CoA) levels in livers of vitamin B_{12}-deficient and supplemented rats to provide further information on the role of this vitamin in metabolism

Some weanling rats from mothers fed diets deficient in vitamin B_{12} and casein were fed similar diets for six weeks while others received supplements of vitamin B_{12} After sacrifice, the CoA content of their livers was determined

The deficient groups showed a definitely smaller weight gain, and other abnormalities characterized by a lower content of liver nucleic acids The CoA level was two to three times greater in the livers of vitamin B_{12} deficient animals than in those of the controls No overlapping in the range of values was noted

American Meat Institute Foundation, and
University of Chicago
Chicago Ill

223 Wong W T and Schweigert B S Role of vitamin B_{12} in nucleic acid metabolism I Hemoglobin and liver nucleic acid levels in the rat J Nutrition 58 231-242, Feb 10, 1956

Authors summary The growth rate liver DNA [desoxyribonucleic acid] and RNA [ribonucleic acid] levels and liver regeneration ability after partial hepatectomy of rats fed an [0 06%] iodinated casein basal diet deficient in vitamin B_{12} were lower than those of supplemented controls

In subsequent experiments an uncomplicated vitamin B_{12} deficiency was produced in rats born from mothers fed the vitamin B_{12}-deficient diet without iodinated casein during gestation and lactation The growth rate and liver nucleic acid composition of these rats fed the basal ration were significantly lower than those for the controls fed supplemented rations A syndrome characterized by presence of porphyrin whiskers and scaly feet was also noted in the deficient rats

The addition of dried liver to the non iodinated casein ration did not improve the growth promoting activity of vitamin B_{12} when the latter was present at a level of 50 mcg /kg ration However inclusion of 4 or 10% of dried liver in the diet of the female rats and 10% of liver in the diet of male rats, significantly increased the hemoglobin content of the blood as compared with that observed when the vitamin B_{12}-deficient diet was fed The significance and implications of these findings were discussed

University of Chicago and
American Meat Institute Foundation
Chicago Ill

224 Register U D Effect of vitamin B_{12} on liver and blood nonprotein sulfhydryl compounds, J Biol Chem 206 705, 1954 (abstr Am J Clin Nutrition 2 373 Sept Oct 1954)

Weanling rats born to mothers maintained on vitamin B_{12}-deficient diets were continued on the deficient diets and their weight gains, liver and blood values for ergothioneine glutathione, and total non protein sulfhydryl groups were compared with those in animals receiving the same diet plus injections of vitamin B_{12} There was a marked decrease largely due to glutathione in the levels of liver and blood sulfhydryl groups in the deficient animals There was no significant effect on the liver or blood ergothioneine

225 Ling C. T and Chow B F The influence of vitamin B_{12} on carbohydrate and lipide metabolism J Biol Chem. 206 797-805 Feb 1954 (abstr Blood 9 930 Sept 1954)

In vitamin B_{12}-deficient rats glucose tolerance tests produced abnormally high blood sugar levels Such rats consistently showed lower blood sugar levels after short periods of fasting but developed hunger diabetes sooner than control animals The phospholipide content of the body tissues of such deficient rats was much less per unit weight than the phospholipide content in the tissues of vitamin B_{12}-treated rats In two patients with pernicious anemia in relapse the phospholipide content of the blood was low but rose in treatment with B_{12} A high carbohydrate-low fat diet supplemented with injections of glucose solution produced in normal rats not only a hyperglycemia but also concomitant decrease in glutathione content of the blood Administration of glutathione produced transient but significant drops in the blood sugar of these animals As vitamin B_{12}-deficiency tends to lower blood glutathione it is suggested that the maintenance of an adequate concentration of glutathione may be one of the roles of vitamin B_{12} It is suggested also that glutathione may be involved in the utilization of carbohydrate Thus it is concluded that vitamin B_{12} plays an important role in carbohydrate and lipide metabolism and that its effect on blood glutathione concentration may be of significance in its role in metabolism

J hns Hopkins University
School of Hygiene and Public Health
Baltimore Md

226 Ling C T and Chow B F Effect of vitamin B_{12} on ribose formation in erythrocytes Federation Proc 13 253 March 1954

Our previous studies indicate that (1) vitamin B_{12}-deficiency results in impaired carbohydrate metabolism and decreased blood glutathione concentration and (2) an interrelationship exists between glucose utilization and glutathione concentration Several investigators have presented evidence implicating vitamin B_{12} in the synthesis of nucleosides or nucleic acids It has been found that whereas thymidine and other desoxyribosides or desoxyribonucleic acid may replace vitamin B_{12} for the growth of certain microorganisms thymine cannot Since thymine differs from thymidine in that it lacks desoxyribose vitamin B_{12} may be concerned with the sugar moiety of the nucleosides or nucleic acids The influence of this vitamin on carbohydrate metabolism and nucleic acid synthesis could be readily comprehensible on this basis In this connection it should be interesting to ascertain whether vitamin B_{12} influences the formation of ribose in tissue cells The first phase of this study concerns the *in vitro* formation of ribose in erythrocytes with glucose as substrate Incubation of erythrocytes in isotonic phosphate buffer containing glucose under aerobic conditions for 2 to 3 hours resulted in a marked increase of ribose in the erythrocytes These results suggest that ribose formation may be an important pathway of glucose utilization in mammalian erythrocytes Red cells from B_{12}-deficient rats showed significant depression of ribose formation and oxygen consumption as compared with erythrocytes from B_{12} repleted or normal animals Addition of reduced glutathione to the incubation mixture restored the normal rate of ribose synthesis and oxygen consumption

Jefferson Medical College
Philadelphia Pa

Johns H pkins School of Hygiene and Public Health
Baltimore Md

227 Meites J and Feng Y S L. Counteraction by vitamin B_{12} of protein catabolic effects of cortisone Federation Proc 13 468-469 March 1954

Some investigations have indicated that large doses of vitamin B_{12} partially

overcome the ability of cortisone to inhibit body hair and thymus growth in young rats fed diets deficient in the vitamin and it has been suggested that the latter may enhance the availability of protein to the organism To test this hypothesis a vitamin B_{12}-deficient diet was fed to rats After 20 or 30 days they were given 200 mcg of vitamin B_{12} per Kg of diet alone or with increasing amounts of cortisone acetate for three successive 10-day periods Cortisone was also administered alone Vitamin B_{12} alone increased body weight and food intake but did not alter daily urinary nitrogen losses Cortisone alone greatly increased urinary nitrogen losses and reduced growth and food intake Vitamin B_{12} completely counteracted these actions of cortisone under ad libitum feeding but was ineffective when food intake was limited to that of rats given cortisone without vitamin B_{12} The authors conclude that vitamin B_{12} may be unable to alter normal urinary nitrogen values but can prevent a cortisone induced increase in nitrogen losses by increasing appetite and consequently the intake of carbohydrate and protein

Michigan State College
East Lansing Mich

228 Aschkenasy, M A La vitamine B_{12} et le métabolisme des protéines [Vitamin B_{12} and the metabolism of proteins] Presse méd 62 1148, Aug 14, 1954

Vitamin B_{12} is not only an erythropoietic substance but also a polyvalent factor taking part in protein metabolism and probably also in fat and carbohydrate metabolism

The vitamin is indispensable to the growth of young animals and to the maintenance of weight of adult animals It improves nitrogen balance favoring certain tissues and organs (blood liver lymphoid organs) more than others

It is difficult to produce avitaminosis B_{12} in animals because of (1) large tissue reserves of this vitamin (2) its synthesis by intestinal microflora and (3) the possibility of its partial replacement by methionine

Vitamin B_{12} seems to have its maximum effects on growth in subjects who are undernourished or on diets deficient in animal protein Therefore deficiencies of both vitamin B_{12} and methionine may be found in the same subject. One of the reasons why antibiotics promote growth is that they promote intestinal synthesis of vitamin B_{12}

Study of the anabolic action of vitamin B_{12} shows that it is extremely complex This factor participates in enzymatic reactions effecting synthesis and in the transfer of labile methyl groups The vitamin also has an important role, in conjunction with folic and folinic acids in the synthesis of nucleosides nucleic acids and nucleoproteids, and it also takes part in the formation of porphyrins and in the metabolism of sulfhydryl compounds

Paris France

229 Charkey, L W, Kano, A. K and Anderson, J A. Effects of fasting on free amino acid levels in chick blood as modified by vitamin B_{12} J Biol Chem 210 627-632 Oct 1954

Authors summary A study of fasting in chicks in the presence and absence of dietary vitamin B_{12} has shown the following

1 A fasting rise in free amino acid of blood is associated with metabolic unavailability of the amino acid precursors

2 Fasting effects on the levels of free amino acids in blood are functions of the age of the experimental chicks up to 6 weeks of age

3 Of five amino acids studied lysine shows the most and leucine the least extensive and enduring (in terms of chick age) rise in blood level due to fasting

4 Vitamin B_{12} functions as a moderator of fasting effects on blood levels of free amino acids This moderating action

is operative in a range of vitamin B_{12} nutritional levels above that required for maximal growth

Col ad Agricultural and Mechanical College Fort Collins Colo

230 **Chang I and Johnson B C The effect of vitamin B_{12} on some aspects of glycine metabolism Arch Biochem & Biophys 55 151 156 March 1955**

The addition of vitamin B_{12} to the diet caused a five-fold increase in incorporation of the α-carbon of glycine into the methyl group of choline while vitamin B_{12} supplementation had no effect on the incorporation of the α-carbon of glycine into serine and aminoethanol

University of Illinois Urbana Ill

231 **Wagle S R and Johnson B C Role of vitamin B_{12} in nucleic acid synthesis Federation Proc 16 401 March 1957**

It is well established that formate glycine serine and the methyl group of methionine serve as precursors in the biosynthesis of purines and pyrimidines which are then incorporated into RNA and DNA. Vitamin B_{12} has been postulated to be involved in this synthesis Formate-C^{14} formaldehyde C^{14} glycine 2-C^{14} serine 3-C^{14} and methionine methyl C^{14} were injected into vitamin B_{12}-deficient and B_{12} normal chicks and baby pigs Similarly glucose-C^{14} uniformly labeled was injected into deficient and normal baby pigs Animals were killed after 4 hours Both RNA and DNA were isolated from the livers and the radioactivity was determined The total radioactivity of the RNA and of the DNA isolated from the formate and formaldehyde injected animals was higher than that of the RNA and DNA isolated from the animals which received the other precursors (glycine serine and methionine) However in no case was any difference found between B_{12}-deficient and the B_{12} normal animals Similarly no difference in activity was found in RNA and DNA isolated from the livers of the pigs (both deficient and normal) injected with radioactive glucose These results suggest that vitamin B_{12} is neither involved directly in the incorporation of 1-carbon precursors into nucleic acids in the conversion of glucose to ribose, nor in the incorporation of ribose into nucleic acids The results obtained were similar for both pigs and chicks

University of Illinois Urbana Ill

232 **Sure B Effect of amino acid and vitamin B_{12} supplements on the biologic value of proteins in rice and wheat J Am Dietet A 31 1232 1234 Dec 1955**

Experiments were carried out on rats to determine the biologic value of the proteins in polished rice processed polished rice and enriched hard wheat flour when vitamin B_{12} and amino acids were included in the diet The only source of protein was the rice or wheat The diet included roughage wheat germ cod liver oils hydrogenated vegetable shortening glucose thiamine riboflavin pyridoxine niacin calcium pantothenate para aminobenzoic acid inositol and choline chloride The amino acids added were L-lysine DL threonine and DL methionine

The most significant observation in this investigation is the remarkable influence of minute doses of vitamin B_{12} when supplemented with amino acids which are deficient in the proteins of milled wheat flour on growth and efficiency of protein utilization Vitamin B_{12} in addition to stimulating greater food intake also produces greater efficiency of protein utilization as evidenced from gains in body weight per gram of food intake Such data suggest that vitamin B_{12} may participate as a coenzyme or activator of intestinal peptidases

thus making more essential amino acids available during digestion for protein synthesis '

University of Arkansas
Fayetteville Ark

233 Kik, M C Nutritional improvement of rice, J Am Dietet A 32 647-650, July 1956

Supplements of lysine threonine and vitamin B_{12} were added to whole milled, and enriched rice, and growth of young rats on this diet was studied The addition of lysine and threonine to their rations produced an average gain of 117 2 Gm, and a protein efficiency ratio of 2 61 Gm When vitamin B_{12} (0 1 mcg) was added, there was an additional increase in weight and protein efficiency ratio

University of Arkansas
Fayetteville Ark

234 Henry, K M and Kon, S K Vitamin B_{12} and protein metabolism, Brit J Nutrition 10 39 1956 (abstr J Am Dietet A 32 658, July 1956)

' The effect of vitamin B_{12} on protein metabolism has been studied in a series of experiments with B_{12}-deficient and normal rats For the deficient rats the biologic value of casein was increased to the same extent by the addition of 1 per cent methionine as by the addition of 1 per cent homocysteine and a vitamin B_{12} supplement Homocysteine was ineffective in the absence of B_{12} It is concluded that vitamin B_{12} is involved in the synthesis of methyl groups

METABOLIC DISTURBANCES (ENDOCRINE)

235 Meites, J and Feng, Y S L Effects of insulin on vitamin B_{12} requirements Federation Proc. 14 100 March 1955

'1) On a vitamin B_{12} deficient diet single injections of insulin reduced blood sugar only about ½ as much in normal and alloxan diabetic rats and only about ⅓ as much in cortisone injected rats as in similarly treated animals fed a vitamin B_{12} abundant diet 2) Insulin injections greatly reduced the urinary excretion of tracer doses of radioactive vitamin B_{12} in normal alloxanized and cortisone-treated rats whether on a vitamin B_{12}-deficient or abundant ration Insulin was least effective in this respect in the cortisone treated vitamin B_{12}-deficient rats 3) In glucose tolerance tests (750 mg injected intraperitoneally, followed by blood glucose determinations 1 and 2 hours later), increases in blood and urinary glucose were at least 7 fold greater in rats fed the vitamin B_{12}-deficient than in rats fed the vitamin B_{12} abundant diet These values were about twice as high in alloxanized and cortisone-injected rats fed the former as compared to the latter diet 4) These results suggest that a) insulin increases requirements for vitamin B_{12} in normal, alloxan-diabetic and most of all in cortisone treated rats b) vitamin B_{12} is essential for normal carbohydrate metabolism confirming the work of Chow et al (1951 54) and c) the action of large doses of cortisone in increasing vitamin B_{12} needs (Meites et al 1951 54) is mediated in part through the pancreas '

Michigan State College
East Lansing Mich

236 Best, W R Leithold, S and David D Urinary excretion of vitamin B_{12} Co^{60} in myxedema J Lab & Clin Med 48 783, Nov 1956 (in Soc. Proc)

Urinary excretion of Co^{60} vitamin B_{12} was studied in a man with marked megaloblastic anemia and myxedema to elicit possible relationships between these diseases Response to vitamin B_{12} therapy had been subnormal Doses of 1 0 or 2 0 mcg of Co^{60} vitamin B_{12} with or without 1 to 3 U S P units of intrinsic factor concentrate orally were given, followed by 1 0 mg vitamin B_{12} parenterally, the percentage of Co^{60} vitamin B_{12} in the 24-hour urine was then measured Four sets of tests made during nine months of thyroid therapy (mostly 120 mg per day) showed

low or low intermediate excretions (under 4 per cent or 6 per cent with 1 0 mcg) Co^{60} vitamin B_{12} excretion remained low after four days of tetracycline

Two other patients with untreated myxedema showed low or low intermediate Co^{60} vitamin B_{12} and intrinsic factor concentrate excretions One received chlortetracycline therapy for four days and the intrinsic factor concentrate test remained low Three other myxedema patients one under treatment showed normal excretions (over 8 per cent with 1 0 mcg)

Megaloblastic anemia in hypothyroidism is rare and has been attributed to chance Tests in 3 hypothyroid patients suggest that, in some cases absorption of vitamin B_{12} is hampered by intestinal block or by intraluminal inactivation of intrinsic factor or of vitamin B_{12} Poor intrinsic factor activity indicates poor secretion or an inactivating substance in the patient s gastric juice Inhibition was demonstrated in one of three aspirates This plus paradoxical and prolonged 72 hour excretions suggests variable regurgitation from the intestine of an inhibitor which delays absorption This inhibitor is probably nonbacterial as antibiotics failed to correct malabsorption Consistent malabsorption despite prolonged therapy could indicate inadequate dosage of thyroid, irreversible change resulting from hypothyroidism or a hypothyroid state secondary to intestinal malfunction

Chicago Ill
Hines Ill

237 Lavate, W V and Sreenivasan A Protective action of vitamin B_{12} in the hyperthyroid rat, Nature 178 804-805, Oct 13 1956

In the hyperthyroid rat there is a marked fall in the concentration of vitamin B_{12} in the circulating blood and in the percentage retained in the blood cells this is prevented by vitamin B_{12} administration

University of Bombay
Bombay India

238 Ralli E P Barbosa F X., Dumm, M E, Beck, E M and Laken, B Prolonged administration of vitamin B_{12} folic acid and calcium pantothenate in patients with diabetes mellitus J Clin Endocrinol & Metab 15 898 July 1955 (in Soc. Proc)

Diabetic patients under observation for several years were the subjects of this study Vitamin B_{12} vitamin B_{12} plus folic acid or calcium pantothenate was administered to study the effects on the course of the disease on the insulin requirement and on blood constituents Vitamin B_{12} did not cause striking change in the plasma or nonprotein sulfhydryl levels (glutathione) Some patients however showed an increased sensitivity to insulin The administration of calcium pantothenate was in some cases associated with a decrease in the fasting levels of blood glucose

New York University
Bellevue Medical Center
New York N Y

OTHER CLINICAL AND EXPERIMENTAL USES

OPHTHALMIC CONDITIONS

239 Becker B Lang C A and Chow, B F Vitamin B_{12} excretion and diabetic retinopathy, Am J Clin Nutrition 1 417-423 Sept-Oct 1953

Urinary excretion of vitamin B_{12} was studied in patients with diabetes mellitus some of whom had retinopathy An intramuscular injection of 50 mcg or 65 mcg was given and the urine was assayed microbiologically for vitamin B_{12} Several patients received radioactive vitamin B_{12} and their urine was measured for radioactivity

Diabetic patients with retinopathy excreted significantly more (average 19 mcg) vitamin B_{12} than nondiabetic sub-

jects (average 9 6 mcg) while diabetic pa tients without retinopathy excreted even less (average 4 2 mcg) than healthy con trols Administration of testosterone de creased excretion of vitamin B_{12} in all diabetics with retinopathy who were tested (Normal humans and diabetics without retinopathy were not studied)

A correlation has been noted between Kimmelstiel Wilson lesions of the kidney and diabetic retinopathy Perhaps the dif ference in vitamin B_{12} excretion by dia betics with and without retinopathy is due to differences in renal function

The Johns Hopkins University
School of Hygiene and Public Health
The Wilmer Institute and
The Johns Hopkins Hospital
Baltimore Md

Chow, B F Vitamin B_{12} and dia betic retinopathy, Mod Med 21 195 Nov 15, 1953 (in Late Reports from Medical Centers)

Blindness with advanced diabetes and old age may be related to inability to absorb vitamin B_{12} Measurement of uri nary excretion of the vitamin after ad ministration of radioactive doses showed that diabetic patients with retinopathy excreted about 19 mcg those without eye lesions 4 2 mcg and healthy subjects 9 6 mcg The patients with early retinitis showed definite need of vitamin B_{12}

Johns Hopkins University
Baltimore Md

Rifkin H The Kimmelstiel Wilson *syndrome and its variants* New York State J Med 53 2947 2950 Dec 15, 1953

This article reviews the clinical features pathogenesis diagnosis and treatment of the disease The author mentions clinical and experimental evidence indicating that relative adrenal hyperfunction possibly associated with vitamin B_{12} deficiency may be related to the development of both glomerular and retinal lesions

Montefiore Hospital
New York N Y

242 Chow, B F , Rosen, D A and Lang C A Vitamin B_{12} serum levels and diabetic retinopathy, Proc Soc Ex per Biol & Med 87 38 39, Oct 1954

Serum levels of vitamin B_{12} were com pared in diabetic patients 16 with and 18 without diabetic retinopathy The pa tients with retinal lesions had much higher serum vitamin B_{12} levels than diabetics without retinal involvement 292±24 micromicrograms per cc and 162±18 micromicrograms per cc respectively

It is stated that diabetics with retinop- athy apparently handle vitamin B_{12} dif ferently from those who do not have the complication and that vitamin B_{12} is in volved in the pathogenesis of the retinop athy The authors mention data which indicate that the hyperglycemia and gly cosuria induced by prolonged injection of cortisone can be corrected by the paren teral administration of vitamin B_{12} These and other data lead to the suggestion that administration of vitamin B_{12} with insu lin cortisone or ACTH might offer clin ical advantages over the administration of these hormones alone

Johns Hopkins University and
Johns Hopkins Hospital
Baltimore Md

243 Bellavia M and Pellegrino, F Vita min B_{12} in ophthalmology, Arch di ottal 56 65 82, Jan Feb 1952 (abstr Am J Ophth 36 1160 Aug 1953)

Vitamin B_{12} was used locally and sys- temically in the treatment of corneal le sions of rabbits The authors report that lesions healed 25 per cent faster in treated animals than in the controls

A case of fascicular keratitis which showed no response to penicillin vitamin D_2 and atropine cleared after vitamin B_{12} had been administered locally and systemically for eight days

Eczematous keratitis in 5 patients and corneal abscess in 1 also showed satisfac tory improvement after treatment with vitamin B_{12}

244 Bellavia M Vitamin B_{12} in high doses in some corneal diseases Arch di ottal 59 517 523 Nov Dec 1955 (abstr Am J Ophth 41 724, April 1956)

Intractable dystrophic or paradys trophic conditions of the cornea in 6 pa tients were effectively treated with local applications of a solution of vitamin B_{12} (500 mcg vitamin B_{12} per cc)

OSTEOARTHRITIS

245 Melendez, E O Júnemann B C and Berrios R Acción de la vita mina B_{12} en las artrosis [Effect of vita min B_{12} in arthritis] Rev méd de Chile 73 76 Aug 1953 (reprint Se mana méd Supl Diar 3130D Jan 6 1954)

Of 32 patients with radiologically con firmed osteoarthritis treated with one to five weekly 100 mcg intramuscular injec tions of vitamin B_{12} 21 made good im provement 6 satisfactory improvement and 5 showed poor results Of the 27 with favorable results 22 were still well a month later 5 received only temporary relief Twenty-eight experienced an in crease in general well being and appetite There were no hematologic changes in the 8 patients studied for them

None of these patients had other rheumatic conditions and any treatment other than vitamin B_{12} had been discon tinued for at least a month before vita min B_{12} was given

246 Norcross B M Lockie L M and Talbott J H Osteoarthritis Gen Practitioner 11 93 101 March 1955

The authors discuss the pathology differential diagnosis and treatment of osteoarthritis and the means used for re lief of pain and swelling Their experience shows that weekly doses of 1 000 mcg of vitamin B_{12} are effective when symptoms are radicular in origin

University of Buffalo and Buffalo General Hospital Buffalo N Y

SERUM LEVELS

247 Unglaub W G Rosenthal H L and Goldsmith G A Studies of vitamin B_{12} in serum and urine fol lowing oral and parenteral adminis tration J Lab & Clin Med 43 143 156 Jan 1954

Comparisons of vitamin B_{12} activity in serum and urine as indicated by micro biologic assays using *L leichmannii* were made after oral and parenteral admini tration of the vitamin The authors con clude Urine vitamin B_{12} activity follow ing oral doses of 500 or 1 000 mcg dif fered little from that prior to administra tion of the vitamin Following doses of 3 000 mcg an increase in urine vitamin B_{12} activity equivalent to that which fol lowed intramuscular injection of 10 mcg of the vitamin was noted in most in stances No definite relationship between maximum total serum vitamin B_{12} activity and urinary excretion of the vitamin was apparent Maximum levels of total serum vitamin B_{12} activity and urinary excretion tended to increase with repeated oral doses of the vitamin No correlation was observed between maximum total serum vitamin B_{12} activity and hematopoietic response

Tulane University School of Medicine and Charity Hospital of Louisiana New Orleans La

248 Pitney W R and Beard M F Serum and urine concentrations of vitamin B_{12} following oral administra tion of the vitamin Am J Clin. Nu trition 2 89 96, March April 1954

The concentration of vitamin B_{12} in the serum of normal subjects and of pa

tients with pernicious anemia, after doses of 1 000 and 5 000 mcg was determined by a method which distinguishes between free vitamin B_{12} and that bound in a heat labile complex with serum protein

The oral administration of 1 000 mcg produced no significant change but with 5 000 mcg both normal subjects and pernicious anemia patients showed increased serum concentrations In patients with pernicious anemia there was a correlation between the finding of vitamin B_{12} in the serum and the hematologic response The authors results confirm other reports that the vitamin B_{12} serum levels are reduced in pernicious anemia It is suggested on the basis of experimental results that the critical serum level necessary for the maintenance of normal erythropoiesis is more than 20 micromicrograms per cc

Vitamin B_{12} administered orally is absorbed from the intestine in bound form and appears in the serum in a different form than when it is given intramuscularly Thus it is impractical to estimate the amount absorbed after oral administration by comparing the amount excreted in the urine with that excreted after smaller amounts given intramuscularly It appears that bound vitamin B_{12} is not excreted in the urine whereas free vitamin B_{12} is so excreted It is suggested that orally administered vitamin B_{12} raises the serum level of bound vitamin B_{12} and does not contribute to free vitamin B_{12} levels to the extent that parenteral vitamin B_{12} does

Unive sity of Louisville
School of Medicine
Louisville Ky

249 Pitney W R, Beard, M F and Van Loon, E J Observations on the bound form of vitamin B_{12} in human serum, J Biol Chem 207 143 152, April 1954 (abstr Blood 9 1208 Dec 1954)

The vitamin B_{12} normally present in human serum is combined with protein primarily It is split off, to a certain extent at least, when serum proteins are denatured by heat A microbiological assay technic employing *Euglena gracilis* distinguishes between free and bound vitamin B_{12} As parenteral injection of more than 80 micrograms of B_{12} has been followed by the rapid urinary excretion of the major part of the dose it has been suggested that there is a limit to the capacity of serum to bind the vitamin Proteins of normal sera were fractionated by paper strip electrophoresis and the vitamin B_{12} concentration measured by microbiological assay Normally most, if not all of the B_{12} was bound to the alpha globulins The addition of crystalline B_{12} to normal sera revealed a limited capacity to bind the vitamin Consistently however the total amount of vitamin recovered' by bioassay did not equal the amount added to the sera This raises the question of whether some technical refinement is needed or whether there is in serum a substance that inhibits the activity of B_{12} in this particular type of assay It is possible megaloblastic anemia may result from a deficiency in vitamin B_{12} binding protein Further studies on correlation of vitamin B_{12} and alpha globulin concentration in sera will be of interest

University of Louisville
School of Medicine and
Veterans Administration Hospital
Louisville Ky

250 Ross, G I M, Mollin D L, Cox E V and Ungley, C C Hematologic responses and concentration of vitamin B_{12} in serum and urine following oral administration of vitamin B_{12} without intrinsic factor, Blood 9 473 488, May 1954

Serum and urine vitamin B_{12} levels (determined by microbiological assay) have been correlated with hematologic effect in pernicious anemia patients receiving vitamin B_{12} by mouth or (in a few cases) rectally Observations were made in 38 patients with pernicious anemia in 2 with megaloblastic anemia following partial gastrectomy and in 4 normal sub-

jects The oral doses ranged from 500 to 3 000 mcg the rectal dose was 1 000 or 3 000 mcg of vitamin B_{12} given in 500 cc of saline

The response to the 500 mcg oral dose of vitamin B_{12} was variable in all subjects With oral doses of 1 000 mcg or 3 000 mcg all patients showed some hematologic response This variable response was associated with a variable rise in serum concentration of vitamin B_{12} The average hematologic effect of 3 000 mcg of vitamin B_{12} by mouth in the pernicious anemia patients was equivalent to that produced by 40 mcg of vitamin B_{12} given intramuscularly The average response to an oral dose of 1 000 mcg was about equivalent to that following 20 mcg intramuscularly

In 14 of 16 patients in whom excretion studies were done the urinary excretion of the vitamin increased after doses of 1 000 or 3 000 mcg of vitamin B_{12} but there was no obvious correlation between the hematologic effect of the dose and the amount of vitamin B_{12} excreted in the first 24 hours The average amount of the vitamin excreted in the urine after the oral doses of 3 000 mcg was only one quarter of the amount excreted after the injection of 40 mcg This lower excretion appears due to the lower serum concentrations of the vitamin and particularly to the lower serum concentration of uncombined vitamin In 3 of 4 normal subjects after doses of 3 000 mcg by mouth there was a rise in vitamin B_{12} levels of both serum and urine

Rectal administration of vitamin B_{12} resulted in adequate hematologic response in all 4 patients receiving 3 000 mcg and in 1 of 3 receiving 1 000 mcg Evidence of absorption (increased serum vitamin B_{12} levels) was obtained in most of these patients

These studies show that intrinsic factor plays little part in absorption of large oral doses of vitamin B_{12} This view is supported by the fact that the average increase in the serum vitamin B_{12} concentration of the normal subjects given 3 000 mcg by mouth was no greater and in fact was slightly less than that of the patients with pernicious anemia Moreover, very large amounts of normal gastric juice would be required even for the absorption of smaller quantities such as 30 to 40 mcg Finally doses of 1 000 or 3 000 mcg of vitamin B_{12} given by rectum where presumably no intrinsic factor was present resulted in similar hematologic effects and changes in serum vitamin B_{12} concentrations

Postgradu te Medical School of London and
Royal Victoria I firmary
Newcastle-upon-Tyne England

251 Mollin D L and Ross G I M Vitamin B_{12} deficiency in the megaloblasticanaemias Proc Roy Soc Med 47 428-431, June 1954

This study presents the results of assays of vitamin B_{12} in the serum of more than 280 patients with various types of megaloblastic anemia Patients with pernicious anemia subacute combined degeneration of the cord total or partial gastrectomy or intestinal anastomosis had low serum levels of vitamin B_{12} Normal levels were found in patients with idiopathic steatorrhea nontropical nutritional megaloblastic anemia and megaloblastic anemia of pregnancy

Vitamin B_{12} given intramuscularly or orally (in small doses with intrinsic factor or in large doses without it) raised the vitamin B_{12} serum levels of patients with pernicious anemia to within the normal range The values remained elevated for varying periods depending on the size of the dose the amount excreted and on the individual requirements of the patient for vitamin B_{12}

The authors conclude that the assay of serum vitamin B_{12} levels (a more sensitive method than ordinary hematologic technics) is useful in detecting vitamin B_{12} deficiency in gastrectomized patients in vegetarians or in relatives of patients with pernicious anemia Study of such

groups should indicate how soon anemia develops after the serum vitamin B_{12} level has fallen below the normal range In some patients with pernicious anemia the serum vitamin B_{12} level has been low but the blood picture normal or almost normal for at least one to two years Serum level assay is also a most convenient method of determining the specific vitamin deficiency in patients with megaloblastic anemia

Postgraduate Medical School of London
London England

252 Beard, M F Pitney W R and Sanneman E H Serum concentrations of vitamin B_{12} in patients suffering from leukemia Blood 9 789 794, Aug 1954

The vitamin B_{12} concentrations of serum were determined in patients with various types of leukemia In 22 normal subjects vitamin B_{12} serum concentrations varied from 86 to 460 micromicrograms per cc In 18 patients with chronic lymphatic leukemia the mean of 209 micromicrograms per cc was close to that of the normals In 12 patients with chronic myeloid leukemia the serum vitamin B_{12} levels were about 15 times greater than the mean of the normals The range for the myeloid leukemia group was from 540 to 6 500 micromicrograms per cc Of 7 patients with acute leukemia 3 had levels of vitamin B_{12} in serum within the normal range and 4 had moderately elevated levels (range 216 to 1 000 micromicrograms per cc, mean 599 micromicrograms per cc)

The present study devoted mainly to sera from patients with chronic myeloid leukemia, showed that vitamin B_{12} was totally in the bound form Electrophoretic studies of sera from 3 patients with myeloid leukemia showed that alpha globulin fractions were the main source of bound vitamin B_{12} This is also the case in normal sera but the binding capacity of this fraction in myeloid leukemic sera is much higher Remissions in myeloid leukemia were accompanied by a fall in serum vitamin B_{12} concentrations but these remained well above normal. During relapses a rise in vitamin B_{12} concentrations in serum occurred In general high white cell counts in myeloid leukemia were associated with higher levels of vitamin B_{12} in the serum

It is postulated (a) that the high serum levels of vitamin B_{12} in myeloid leukemia may be associated with myeloid activity (b) that in the disease alpha globulin is qualitatively different in its ability to bind the vitamin and (c) that the high vitamin B_{12} levels may reflect decreased erythroid activity

University of Louisville
School of Medicine
Louisville Ky

253 Beard, M F Pitney W R, Sanneman E H, Sakol, M J and Moorhead H H Serum concentrations of vitamin B_{12} in acute leukemia Ann Int Med 41 323 327, Aug 1954

Serum levels of vitamin B_{12} were determined in 56 normal persons and in 20 patients with various types of acute leukemia Patients with acute lymphocytic leukemia had normal vitamin B_{12} serum concentrations those with acute myelocytic leukemia had mean concentrations of about 13 times normal and the patients with acute monocytic leukemia had moderately elevated levels There was no correlation in any of the leukemia types between the serum vitamin B_{12} concentration and the total white cell count

In the discussion the authors comment In vitro normal serum will bind only from 208 to 576 µmcg /ml of vitamin B_{12} when excess free vitamin is added In the cases of acute myelocytic leukemia studied the mean concentration of bound vitamin was found to be 2 570 µmcg /ml The results demonstrate that the serum protein responsible for vitamin B_{12} binding is abnormal in acute myelocytic leukemia It is possible that the binding protein is liberated into the serum by the disintegration of myeloid

cells The lack of correlation between the serum vitamin B_{12} concentrations and white cell counts would make this appear unlikely Our results suggest that vitamin B_{12} therapy would be of no avail in the treatment of acute leukemia as in no case were serum concentrations found below normal

Louisville Ky

254 Lear A A Harris J W, Castle W B and Fleming E M The serum vitamin B_{12} concentration in pernicious anemia J Lab & Clin Med 44 715 722 Nov 1954

The results of microbiologic assays of serum vitamin B_{12} levels using *Euglena gracilis* are reported The mean total serum vitamin B_{12} level in 20 normal subjects was 532 micromicrograms per cc all had free vitamin B_{12} present In 33 patients with pernicious anemia in relapse the mean level was 39 micromicrograms per cc. and no free vitamin B_{12} was detected Twelve patients with megaloblastic anemia that subsequently responded to folic acid therapy all had levels within the normal range and a mean level of 307 micromicrograms per cc

In 5 patients with hepatic cirrhosis the mean vitamin B_{12} serum level was 714 micromicrograms per cc most of which was in bound form None had received therapy with vitamin B_{12} It is possible that the serum of these patients possesses increased vitamin B_{12} binding capacity since the globulins are characteristically elevated and abnormal in such patients

Harvard Medical S hool and Bo ton City Ho pital Boston Ma

255 Wilson H E and Pitney W R Serum concentrations of vitamin B_{12} in normal and nutritionally deficient monkeys J Lab & Clin Med 45 590-598 April 1955

The vitamin B_{12} serum levels of monkeys fed a diet deficient in vitamin B_{12} and folic acid showed a gradual decline Addition of folic acid and 2 mcg of vitamin B_{12} caused little change but 10 or 20 mcg of vitamin B_{12} caused a rise to high normal levels

Animals with severe folic acid deficiency showed normal vitamin B_{12} levels which increased during recovery from the folic acid deficiency In animals with ascorbic acid deficiency low vitamin B_{12} levels were seen The vitamin B_{12} rose to a high level during recovery from this deficiency The authors state that ascorbic acid may be influential in absorption of vitamin B_{12} and folic acid The studies also indicated that the monkey is able to store a considerable amount of vitamin B_{12} The comparison between the serum vitamin B_{12} concentrations in monkeys and in man is not conclusive since greater amounts of the free vitamin are found in monkey serum than in human serum

Northweste n Uni e sity Medical School Evanston Ill

Uni ersity of Louisville Louisville Ky

256 Lear A A Effect of folic acid on serum vitamin B_{12} concentrations in pernicious anemia J Clin Investigation 34 948 June 1955 (in Soc Proc)

Therapy with folic acid alone sometimes has a neuropathic effect in pernicious anemia A study was therefore made of the effect of folic acid on serum vitamin B_{12} concentrations in the disease

Twenty seven patients with pernicious anemia who had been receiving 15 mcg of vitamin B_{12} intramuscularly every four weeks for two or more years were selected During the first six months 20 were given 30 mcg and 7 were given 20 mcg of vitamin B_{12} intramuscularly every two weeks During the next six months all received the same vitamin B_{12} dosage and in addition took 5 mg of folic acid by mouth daily Serum vitamin B_{12} levels were estimated microbiologically initially

and after 6 and 12 months using *Euglena gracilis*

Initially 14 of the 27 patients had serum vitamin B_{12} levels of less than 100 after the first six months only 1 patient had a level of less than 100 and the average was 451 After the second six months of supplemental folic acid the average level was 279 At this time, individual serum vitamin B_{12} levels in 18 of the 27 patients were at least 15 per cent lower than after the first six months but no neuropathic effects were observed Results indicate that injection of 15 mcg of vitamin B_{12} every four weeks may be insufficient to saturate body vitamin B_{12} stores Administration of folic acid may lower the serum level of vitamin B_{12} possibly by accelerating its utilization

Boston Mass

7 **Das Gupta, C R, Chatterjea, J B, Ghosh, S K. and Banerjee D K Vitamin B_{12} concentration of serum in nutritional macrocytic anaemia Bull Calcutta School Trop Med 3 101, 1955 (abstr Blood 11 688-689 July 1956)**

Mean values for total and combined concentrations of vitamin B_{12} in serum in 6 cases of nutritional macrocytic anaemia estimated microbiologically with *Euglena gracilis* var *bacillaris* as the test organism were respectively 75 (range 20 to 170) and 72 (range 20 to 150) µmcg per cc These *values were significantly lower than the* corresponding values in normal Indians After treatment with specific antimegaloblastic drugs the values of both combined and total vitamin B_{12} attained normal levels The findings suggest that in an average case of nutritional macrocytic anaemia as seen in India there is deficiency of vitamin B_{12}

School of Tropical Medicine
Calcutta India

8 **Foy, H, Kondi A and Manson Bahr, P E C Penicillin in megaloblastic anaemias of Africans Effect on serum vitamin B_{12} levels and absorption of radioactive vitamin B_{12}, Lancet 2 693-699, Oct 1, 1955**

Nonpernicious megaloblastic anemia was studied in African natives Red cell counts were 600 000 to 2,000 000 per cu mm and reticulocyte counts 1 per cent when the patients were admitted The patients were observed for 3 to 10 days then serum vitamin B_{12} levels and excretion of radioactive vitamin B_{12} were studied The patients were then given 400 000 units of penicillin intramuscularly daily for 6 to 12 days, if there was no response 80 mcg of vitamin B_{12} was given intra*muscularly or orally If no response was* yet seen folic acid was administered orally The authors state that this order of giving medication is important since all cases respond to folic acid but not all respond to vitamin B_{12} or to penicillin

The patients who responded completely to penicillin or to vitamin B_{12} had low vitamin B_{12} serum levels (20 to 100 micromicrograms per cc) before treatment The authors found that penicillin increased the serum vitamin B_{12} levels but were uncertain if this increase was due to improved absorption, to reduction of the organisms competing for vitamin B_{12} or to metabolic changes in the host

Patients responding to folic acid had normal or high vitamin B_{12} serum levels before treatment (300 to 1,300 micromi*crograms per cc) Patients with serum* levels below 700 micromicrograms who eventually improved with folic acid had also shown a slight reticulocytosis after penicillin

Diets in Africa are generally low in animal protein and rich in carbohydrates but it is not known whether they are deficient in vitamin B_{12} or folic acid or in both The diet may encourage growth in the gastrointestinal tract of bacterial competitors for vitamin B_{12} At a certain time of year the anemia that responds to penicillin is predominant, while at other times

the type that responds to folic acid predominates

Wellcome Trust Research Laboratories and
Kenya Medical Service
Kenya Colony East Africa

259 Murata K. and Miyamoto T The determination of vitamin B_{12} activity in human blood J Vitaminol 1 297 304 Oct 10 1955

Authors summary The procedures for releasing and extracting vitamin B_{12} from proteins in whole blood were investigated by the microbiological assay using *Lactobacillus leichmannii* ATCC 4797

The treatment of the whole blood by *heating with potassium cyanide was found* to give constant values at each level of the vitamin, and good recoveries of the added vitamin in the assay

The individual values of the vitamin in whole blood estimated by heating with potassium cyanide are in agreement with those obtained by using sodium meta bisulfite and those by papain digestion but not with those by digestion with the trypsin preparation available in this country

The vitamin B_{12} content of healthy human whole blood was found to range from 0 10 to 0 66 mcg /cc the average value being 0 28±0 05 (U S.A) and 0 37 ±0 07 mcg /cc (Japan)

Osaka City University
Nishi-ku Osaka Japan

260 Dhopeshwarker G A Trivedi J C Kulkarni B S Satoskar R. S and Lewis R. A Blood proteins and vitamin B_{12} in vegetarians Brit J Nutrition 10 105 1956 (abstr J Am Dietet A 32 844-846 Sept 1956)

Plasma protein and blood vitamin B_{12} were determined in five Indian medical students on a vegetarian diet which included milk and milk products but no other food of animal origin and in five students whose diet included eggs fish fowl or meat daily The vegetarians were given 50 mg Aureomycin daily for two months and determinations were repeated after one or two months The albumin content of the plasma was below normal and the globulin content above normal in the vegetarians who also had a lower blood content of vitamin B_{12} The administration of Aureomycin to the vegetarians produced a rise in plasma albumin and a fall in all the globulin fractions Values for blood vitamin B_{12} increased significantly

261 Miller A Corbus H F and Sullivan J F The plasma disappearance excretion and tissue distribution of cobalt[60] labelled vitamin B_{12} in normal subjects and patients with chronic myelogenous leukemia J Clin Investigation 36 18-24 Jan 1957

The plasma disappearance tissue distribution and excretion of a 4 mcg intravenous dose of Co^{60} vitamin B_{12} were studied in control subjects and in patients with leukemia The dosage used is about four times the binding capacity of normal serum but is within the total binding capacity of chronic myelogenous leukemia serum

Plasma radioactivity declined rapidly in the 7 normal subjects in 1 patient with chronic myelogenous leukemia in remission and in 1 patient with chronic lymphocytic leukemia The 7 patients with chronic myelogenous leukemia however showed a slow decline in plasma radioactivity while 2 with myeloid metaplasia showed an intermediate plasma disappearance rate Normal subjects excreted 1 to 4 per cent of the administered dose in the urine within 24 hours compared with 0 to 2 per cent excreted by chronic myelogenous patients Stool collections from 2 normal subjects showed no radioactivity In 1 patient with cirrhosis the bile contained 2 per cent of the dose and the ascitic fluid none

Normal subjects showed an increase

in liver radioactivity throughout the period of observation which was not associated with a comparable fall in plasma radioactivity Radioactivity was not concentrated in the spleen or white cells of leukemia patients

The serum vitamin B_{12} concentration ranged from 364 to 530 micromicrograms per cc in 3 normal subjects and from 2 046 to 9,266 micromicrograms per cc in 4 patients with chronic myelogenous leukemia It was 704 in a patient with myeloid metaplasia and 2 104 in the patient with cirrhosis There was no correlation between the leukocyte count and the serum vitamin B_{12} levels

The increased vitamin B_{12} binding power of the plasma of patients with active chronic myelogenous leukemia may aid in differentiating this leukemia from leukemoid states

Boston University School of Medicine
Tufts University School of Medicine and
Boston Veterans Administration Hospital
Boston Mass

262 Grossowicz, N, Hochman A, Aronovitch J Izak G and Rachmilewitz M Malignant growth in the liver and serum vitamin B_{12} levels, Lancet 1 1116-1117, June 1, 1957

Serum vitamin B_{12} levels were measured in 35 healthy people in 18 patients with tumors and metastases of the liver and in 19 with malignant diseases not involving the liver

Serum values of normals ranged from 200 to 500 micromicrograms per cc (assay of *Esch coli)* The 19 patients without liver metastases had normal serum vitamin B_{12} levels (100 to 550 micromicrograms per cc) However 16 of the 18 patients with liver metastases had raised serum vitamin B_{12} levels of 640 to 20,000 micromicrograms per cc The highest level occurred in a patient with total gastrectomy jaundice and extensive liver metastases One patient with multiple myeloma and extensive amyloidosis had raised vitamin B_{12} levels while 2 patients with multiple myeloma not complicated by amyloid had normal levels of vitamin B_{12} In 1 patient with hepatoma cirrhosis and a raised serum vitamin B_{12} level autopsy revealed extensive infiltration of the liver In another patient with hepatoma and a normal serum vitamin B_{12} level, the tumor was limited to a circumscribed area

A tumor invading the liver by causing necrosis and displacement of liver cells causes the stored vitamin B_{12} to be released into the blood Because the process in cancer is prolonged a persistent rise is to be expected and was in fact found in *3 patients in whom estimations were re*peated throughout the illness

These findings suggest that determination of the serum vitamin B_{12} may help the diagnosis of metastases to the liver in cases of malignancy Raised levels should be interpreted as an indication that the liver is involved Normal levels however, do not rule out this possibility, because metastasizing tumours not causing liver damage could be present in the liver without raising the serum vitamin B_{12} level

Hebrew University
Hadassah Medical School
Jerusalem Israel

263 Bertcher, R W, Meyer L M and Cronkite, E P Serum Co^{60} vitamin B_{12} binding capacity in some hematologic disorders Clin Research Proc 5 143 144 April 1957

It has been recently reported that the range of Co^{60} vitamin B_{12} binding capacity of normal human serum may be estimated by incubating the radioactive vitamin with serum and dialyzing against running tap water for 48 hours This binding capacity has been studied in various hematologic disorders with the following results

Ten of 13 cases of chronic myelocytic leukemia showed an elevated binding ca

pacity which was roughly but not regularly proportional to total leukocyte count. Those cases showing normal binding were under treatment and in remission. One of six cases of chronic lymphocytic leukemia showed moderate and two slight increase of binding capacity. This varied with leukocyte count but was less elevated than in chronic myelocytic leukemia. Increased binding capacity was also found in one of seven adult cases of acute leukemia, two cases of chronic idiopathic leukopenia, one of two cases of polycythemia vera and none of three cases of pernicious anemia in relapse.

The findings in chronic myelocytic leukemia and polycythemia vera parallel reports of increased serum content of vitamin B_{12} in these diseases.

In none of the conditions studied was binding capacity reduced.

*South Nassau Communities Hospital
Oceanside, N. Y.*

*Brookhaven National Laboratory
Upton, N. Y.*

264 Bertcher, R. W. and Meyer, L. M. Co^{60} vitamin B_{12} binding capacity of normal human serum. Proc. Soc. Exper. Biol. & Med. 94: 169-171. Jan. 1957.

This study presents a method of estimating the Co^{60} vitamin B_{12} binding capacity of human serum with data on 40 normal subjects. As the amount of Co^{60} vitamin B_{12} added to the serum was increased, the binding capacity of the serum increased. The higher binding capacity shown by this method of measurement compared to that using microbiological assay is not due to ionic cobalt. The discrepancy occurs because the amount of vitamin B_{12} made available to a microorganism by heating serum does not represent the total vitamin bound to the serum proteins. The vitamin is bound to alpha globulin at usual body levels but at higher concentrations this mechanism may be saturated and nonspecific binding to other serum protein fractions may take place.

*South Nassau Communities Hospital
Oceanside, N. Y.*

265 Sheely, L. L., Miller, O. N. and Unglaub, W. G. Vitamin B_{12} levels in serum and urine following parenteral administration of crystalline vitamin B_{12} or liver extract. Proc. Soc. Exper. Biol. & Med. 94: 629-631. April 1957.

Serum and urine levels of vitamin B_{12} were determined in normal subjects, 8 of whom received 50 mcg. of crystalline vitamin B_{12} parenterally and 8 of whom were given an equivalent amount of vitamin B_{12} in the form of purified liver extract. Blood samples were drawn before injection and 1, 4, 8 and 24 hours after injection. Urine was collected for 24 hours after injection. Total serum vitamin B_{12} levels, bound vitamin B_{12} levels and urinary excretion of vitamin B_{12} were compared in the two groups.

The average maximum serum concentration of total vitamin B_{12} was 1.8 mcg. in the group receiving crystalline vitamin B_{12} compared to 1.2 mcg. in the group receiving liver extract. Concentration of bound vitamin B_{12} was 0.8 mcg. in both groups. Maximum serum concentration of vitamin B_{12} was reached faster in subjects receiving crystalline vitamin B_{12}. Calculation of the average proportion of bound vitamin to total vitamin over a 24 hour period demonstrated that about 81.5 per cent of vitamin B_{12} was bound following administration of liver extract compared to 64.9 per cent after injection of crystalline vitamin B_{12}. Urinary excretion of vitamin B_{12} in the group receiving crystalline vitamin B_{12} averaged 9.0 ± 0.75 mcg. compared to an average excretion of 6.0 ± 1.1 mcg. in the group receiving liver extract. This was considered significantly lower. It was attributed to the lower proportion of free vitamin B_{12} present after administration of liver extract.

*Tulane University School of Medicine
New Orleans, La.*

time needed for depletion of stores of vitamin B_{12} and development of macrocytic anemia after total gastrectomy as well as long remissions observed in pernicious anemia following treatment with liver extracts or vitamin B_{12}

New York Medical College
Flower and Fifth Avenue Hospitals
New York N Y

269 Turnbull A Experiences with labelled vitamin B_{12} Proc. Roy Soc Med. 47 424-426 June 1954

Between 20 and 40 per cent of orally administered radioactive vitamin B_{12} (0 5 mcg) was recovered from the feces of 10 control subjects compared to an 80 to 95 per cent recovery from 22 patients with pernicious anemia in remission Since nearly all the pernicious anemia patients were receiving injections of vitamin B_{12} it was concluded that perhaps they were excreting large amounts of the radioactive preparation because their tissues were already saturated with the vitamin Radioactive vitamin B_{12} was therefore given with intrinsic factor to 7 patients with pernicious anemia In these 7 patients recovery of radioactivity was much reduced and with the more potent intrinsic factor preparations the recoveries approached those found in the control patients It was concluded that the difference between the findings in the control subjects and the patients with pernicious anemia was due to the failure of the latter to secrete intrinsic factor

The same lack of intrinsic factor apparently occurs after total gastrectomy indicating that in man the stomach is the only gastrointestinal source of intrinsic factor

Normal recoveries were made in 3 patients with steatorrhea who apparently had normal secretion of intrinsic factor and absorption of vitamin B_{12} In 2 patients however the recoveries were greater than normal but were not reduced by the addition of intrinsic factor Probably the defect in absorption in these patients was not due to lack of intrinsic factor In 1 patient the recovery was similar to that from patients with pernicious anemia and it was greatly reduced when intrinsic factor was also given

In 2 patients with megaloblastic anemia of pregnancy (both receiving folic acid) the recoveries of radioactivity were within normal limits Therapy with folic acid may have caused resumption of the secretion of intrinsic factor in these patients or its return to normal levels though it does not have this effect in addisonian pernicious anemia

The drawbacks of this technique of estimating vitamin B_{12} excretion and absorption are pointed out but it is stated that it nevertheless appears to provide a useful indirect measure of the secretion of intrinsic factor

The Radcliffe Infirmary
Oxford England

270 Krevans J R Conley C L and Sachs M Influence of certain diseases on the absorption of vitamin B_{12} from the gastrointestinal tract J Clin Investigation 33 949 950 June 1954 (in Soc Proc)

In 16 normal individuals administration of 0 1 to 0 5 mcg of Co^{60} vitamin B_{12} was followed by recovery of 10 to 47 per cent of the radioactivity in the stool Within this range there was no relationship between the amount of vitamin B_{12} administered and the per cent recovered With a dose of 1 0 mcg as much as 60 per cent of the radioactivity was recovered and with 5.5 mcg more than 70 per cent In 23 studies on patients with achlorhydria liver disease or anemia unrelated to vitamin B_{12} deficiency administration of 0 1 to 0 5 mcg of labelled vitamin B_{12} was followed by recovery of 10 to 45 per cent One 21 year-old man with hepatitis and normal gastric acidity excreted 67 and 68 per cent in two studies

In 8 patients with pernicious anemia recovery of radioactivity was more than 60 per cent after a dose of 0 5 mcg vitamin B_{12} One patient who appeared to

have typical pernicious anemia excreted less than 35 per cent and the Schilling test indicated apparently normal vitamin B_{12} absorption One of 2 patients with total gastrectomy and 2 of 3 with sprue showed impaired absorption One patient with megaloblastic anemia subacute combined degeneration normal gastric acidity and multiple diverticula of the jejunum demonstrated markedly impaired absorption which became normal during Aureomycin administration Terramycin failed to produce *normal absorption in a patient with* pernicious anemia

Baltimore Md

271 Glass G B J, Boyd, L J and Stephanson L Intestinal absorption of vitamin B_{12} in man Science 120 74-75 *July 9, 1954* (in Correspondence)

For the purpose of studying the absorption of vitamin B_{12} following parenteral and oral administration the authors used surface scintillation measurements for the uptake of radioactive vitamin B_{12} *by the liver These studies demonstrated* an inverse relationship between the radioactivity counts over the liver and the amounts of crystalline vitamin B_{12} added *to the radioactive vitamin B_{12} ingested*

The intestinal absorption of vitamin B_{12} under normal conditions is apparently controlled by a partial mucosal block *similar to that regulating intestinal iron* absorption This partial mucosal block changes to a complete or almost complete block in sprue and in pernicious anemia *This is indicated by decreased or* absent hepatic uptake of orally administered vitamin B_{12} in these diseases In sprue the block cannot be corrected by the addition *of intrinsic factor* because the absorption mechanism of the intestinal wall is inherently defective In pernicious anemia however the block can be converted into a *partial block by ad*ministration of normal human gastric juice or intrinsic factor concentrate

Merely increasing greatly the intake of vitamin B_{12} overcomes the block and results in some absorption However the principle of regressing efficiency of vitamin B_{12} absorption in the *intestine with* increase of the intake still applies

New York Medical College
Flower and Fifth Avenue Hospitals
New York N Y

272 Halsted, J A, Gasster M and Drenick E J Absorption of radioactive vitamin B_{12} *after total gastrectomy*, New England J Med. 251 161-168, July 29, 1954

The absorption of orally administered, radioactive vitamin B_{12} was studied by using a scintillation counter to determine its fecal excretion In 11 normal persons excretion on the average was 33 per cent of a 0 5 mcg dose in 3 patients with idiopathic achlorhydria the average was 34 per cent In 7 patients with pernicious anemia the average excretion was 93 per cent in 10 tests When intrinsic factor was administered with the test dose in 4 of these patients fecal excretion was decreased to an average of 38 per cent The average excretion in 11 totally gastrectomized patients was 87 per cent in 16 tests When a source of intrinsic factor was administered with the test dose an average of only 20 per cent was excreted in 14 tests

None of the 11 gastrectomized patients *had macrocytic anemia,* but 9 had received therapy to prevent it However 10 were significantly underweight 7 had normocytic anemia and 10 had steatorrhea

Reasons why macrocytic anemia rarely follows total gastrectomy are (1) Few patients survive long enough for anemia to develop (2) liver stores of vitamin B_{12} *may be sufficient for several years* (3) in many operations considered total gastrectomies part of the cardia which secretes intrinsic factor remains and (4) *most patients receive prophylactic anti*anemic therapy

Los Angeles Calif

273 Bradley J E Smith E L Baker S J and Mollin D L The use of the radioactive isotope of cobalt Co^{58} for the preparation of labelled vitamin B_{12} Lancet 2 476-477, Sept 4 1954

Co^{58} has a half life of only 72 days compared to a half life of 5 years for Co^{60} It is therefore suggested for reasons of safety that Co^{58} instead of Co^{60} be used in clinical excretion studies of vitamin B_{12}

Postgraduate Medical School of London London England

Glaxo Laboratories Greenford Middlesex England

Franz, W and Pendl I Influence of bacterial flora of stomach on absorption of orally administered vitamin B_{12} Klin. Wchnschr 32 1092 1096 Dec. 1 1954 (abstr J A M A 157 57 March 12, 1955)

The gastric juice of 3 patients with severe untreated pernicious anemia contained no intrinsic factor or bound vitamin B_{12} The authors were at first unable to culture colon bacilli from the gastric juice of these patients They all had not only a moderate increase in reticulocytes but also changes in the bone marrow suggesting the onset of a spontaneous remission Two of the patients were given small doses (5 10 and 30 mcg) of vitamin B_{12} orally The condition of one did not improve and it was found that her gastric juice contained paracolon bacilli which completely consumed or destroyed vitamin B_{12} (added *in vitro*) In the second patient complete remission of the pernicious anemia resulted but only after treatment for 98 days This patient had paracolon bacilli in his gastric juice only for a short time and *in vitro* tests revealed that these bacilli consumed or destroyed only 80 per cent of the added vitamin B_{12} Colon bacilli found in the gastric juice of the third (untreated) patient destroyed 50 per cent of added vitamin B_{12} More recently the authors have been able to alleviate pernicious anemia in another patient by small oral doses of vitamin B_{12} This patient had no colon bacilli in his gastric juice

The authors believe that vitamin B_{12} taken in food or in small oral doses can be absorbed even if intrinsic factor is absent provided the stomach does not contain colon bacilli that consume or destroy the vitamin

Berlin Germany

275 Glass G B J Pack, G T and Mersheimer, W L Uptake of radioactive vitamin B_{12} by the liver in patients with total and subtotal gastrectomy Gastroenterology 29 666-683 Oct 1955

Intestinal absorption of vitamin B_{12} was studied by direct measurement of the hepatic uptake of Co^{60} vitamin B_{12} In normal subjects ingestion of radioactive vitamin B_{12} results in accumulation of radioactivity in the liver In patients with pernicious anemia radioactivity accumulates in the liver only if intrinsic factor is given with the oral vitamin B_{12} The hepatic uptake of vitamin B_{12} by 7 of 8 subtotally gastrectomized patients was at the lower level of the normal range and above the normal mean in 1 When intrinsic factor was given with oral vitamin B_{12} to 4 of these patients hepatic uptake doubled or tripled in 3 but changed little in the patients whose uptake of the vitamin alone was above the normal mean

The 8 totally gastrectomized patients showed hepatic uptake of oral vitamin B_{12} only when intrinsic factor was given

Presumably the prolonged hepatic storage of vitamin B_{12} explains the belated appearance of anemia after total gastrectomy When radioactive vitamin B_{12} was given intramuscularly to normal subjects or to patients with pernicious anemia or sprue 65 to 86 per cent of the peak radioactivity persisted in the liver two or three months after injection The long interval between total gastrectomy

several years (2) gastrectomy may have been incomplete or (3) the patient may have been given liver extract or vitamin B_{12} prophylactically Evidence indicates that every patient who does survive long enough without prophylactic therapy will develop megaloblastic anemia indistinguishable from addisonian pernicious anemia A monthly injection of 30 mcg of vitamin B_{12} to prevent anemia is recommended

The 3 patients with megaloblastic anemia showed high fecal excretion of the test dose of vitamin B_{12} this did not change with administration of intrinsic factor Chlortetracycline or tetracycline therapy however resulted in a significant decrease in vitamin B_{12} excretion (increased absorption) Bacterial competition for vitamin B_{12} may have been the mechanism involved in producing the anemia An injection of 30 mcg of vitamin B_{12} once a month in such patients controls the deficiency causing the anemia

Veterans Administration Center and
University of California
School of Medicine
Los Angeles Calif

279 Schilling R. F Clatanoff, D V and Korst D R. Intrinsic factor studies III Further observations utilizing the urinary radioactivity test in subjects with achlorhydria pernicious anemia or a total gastrectomy J Lab & Clin Med 45 926-934 June 1955

To test absorption of vitamin B_{12} the radioactive vitamin was administered by mouth to 97 subjects and followed by an intramuscular injection of nonradioactive vitamin B_{12} measurements of urinary radioactivity were then made

The 18 control subjects excreted from 7 to 22 per cent of the radioactivity in 24 hours (average 14.2 per cent) In 31 achlorhydric subjects with no history of pernicious anemia excretion ranged from 2 2 to 29 per cent (average 11 6 per cent) The 35 patients with pernicious anemia excreted 0 to 2 3 per cent when the vitamin was given without intrinsic factor When the same dose of the radioactive vitamin was given with intrinsic factor, these patients excreted from 3 1 to 30 per cent (average 9 8 per cent) The 13 totally gastrectomized subjects were entirely similar to pernicious anemia patients in their ability to absorb vitamin B_{12}

The author states that the urinary radioactivity test is useful in diagnosis of pernicious anemia and in estimating activity of intrinsic factor preparations

University of Wisconsin Medical School
Madison Wis

280 Meyer L M Berlin N I Jiminez Casado M and Arkum S N Vitamin B_{12} distribution determined by surface body counting following parenteral administration of Co^{60} vitamin B_{12} Proc Soc Exper Biol & Med 91 129 131 Jan 1956

Co^{60} vitamin B_{12} 1 mcg was given intramuscularly to 2 normal subjects and to 12 patients with various diseases (arteriosclerosis rheumatoid arthritis cirrhosis pernicious anemia Gaucher s disease lymphosarcoma or polycythemia) Radioactivity was measured over several body sites beginning 30 minutes after the labeled vitamin B_{12} was injected

No significant radioactivity following Co^{60} vitamin B_{12} injection was found over the precordium sacrum or thighs Radioactivity was always detectable over the liver spleen and left kidney within 30 minutes after injection Levels were highest over the liver Radioactivity disappeared from the site of injection within three to four hours

Six subjects were given $Co^{60}Cl_2$ (radioactive cobalt chloride) intramuscularly The pattern of absorption from the injection site and the distribution over the liver spleen and kidney were entirely different from those following Co^{60} vitamin B_{12} administration Thus in studies with Co^{60} vitamin B_{12} it is probable that

the radioactivity measured represents vitamin B_{12} with Co^{60} still attached

Goldwater Memorial Hospital
New York N Y
University of California
Berkeley Calif

281 Unglaub, W G, Miller, O N and Goldsmith, G A Saturation studies with vitamin B_{12} in human subjects Federation Proc 15 374 March 1956

Normal subjects patients with macrocytic anemia and a group of patients with miscellaneous diseases were given vitamin B_{12} 50 mcg intramuscularly daily for 10 days followed by 1000 mcg daily for an additional 10 days After a 3 day interval, a final test dose of 50 mcg was administered Urinary excretion of the vitamin and concentrations of free and bound vitamin B_{12} in serum were measured at suitable intervals There was a significant increase in the concentration of free and bound vitamin B_{12} during the period of saturation in all subjects which in most instances was still present 3 days after the saturation period In all patients with pernicious anemia tested thus far and in some patients with other types of macrocytic anemia the maximum level of bound vitamin B_{12} was significantly less than that attained in normal subjects or in patients with miscellaneous diseases During the period of administration of 50 mcg of the vitamin urinary excretion increased progressively for the first 5 days after which it was maintained at approximately a constant level in most subjects Excretion varied widely among individuals and among groups at all levels of saturation No correlation was observed between urinary excretion of the vitamin and concentration of free bound or total vitamin B_{12} in the serum

Tulane University
New Orleans La

282 Best W R Landmann W A and Limarzi, L R Time pattern of vitamin B_{12} Co^{60} urinary excretion in man after oral administration and parenteral flushing' Blood 11 352 356 April 1956

Serial urine collections in 8 patients with pernicious anemia given 2 mcg Co^{60} vitamin B_{12} orally followed in two hours by 1 000 mcg nonradioactive parenteral vitamin B_{12} showed little radioactivity When the same doses were repeated with a potent oral dose of intrinsic factor concentrate peak excretion of radioactivity usually occurred between 6 and 12 hours after ingestion of radioactive vitamin B_{12}, sometimes even later The excretion rate dropped relatively low after 12 to 24 hours in most patients Similar curves were seen in controls given no intrinsic factor Secondary peaks occasionally occurred

University of Illinois
College of Medicine and
Armour Laboratories
Chicago Ill

283 Miller A, Corbus, H F and Sullivan, J F The plasma disappearance excretion and tissue distribution of intravenous Cobalt^{60} vitamin B_{12} in normal subjects with chronic myelogenous leukemia, J Clin Investigation 35 724, June 1956 (in Soc Proc)

After 4 mcg of Co^{60} vitamin B_{12} was injected intravenously into normal subjects and leukemic patients its disappearance from the plasma tissue distribution and urinary and fecal excretion were determined A rapid decline in plasma radioactivity occurred in normal subjects 7 to 12 per cent of the dose remained at 2 hours and 4 to 10 per cent at 24 hours Urine and stools contained negligible amounts of radioactivity In 7 out of 8 patients with myelogenous leukemia plasma radioactivity disappeared more slowly 50 to 63 per cent of the dose remained at two hours and 38 to 44 per cent at 24 hours After 24 hours the disappearance rate decreased (half time of five days) One patient in remission from chronic myelogenous leukemia 2 with

myeloid metaplasia and 1 with chronic lymphocytic leukemia had normal rates of disappearance

External monitoring showed an increase in liver radioactivity throughout the period of observation (7 to 22 days) not associated with a comparable fall in plasma radioactivity The leukemic patients showed a smaller rise in hepatic radioactivity than did the normals In 2 myelogenous leukemic patients the liver at autopsy contained 39 to 42 per cent of the administered dose the spleen 8 to 11 per cent and the other viscera less than 1 per cent Red and white cells from both groups of patients contained no radioactivity

The increased *in vivo* plasma binding of vitamin B_{12} in myelogenous leukemia without preferential concentration in leukemic tissue suggests that the turnover of vitamin B_{12} in leukemic tissue is not increased The decreased plasma disappearance may prove to be of diagnostic value in differentiating chronic myelogenous leukemia from leukemoid states

Boston Mass

284 Diet and anemia J A M A 161 474 June 2 1956 (in Foreign Letters United Kingdom)

At a symposium of the Nutrition Society Dr F Wokes reported on a sect of 700 vegetarians who eat no animal food whatsoever Their protein intake is only about 10 to 12 Gm daily Although their hematograms are not like those of pernicious anemia these patients do suffer from subacute combined degeneration of the spinal cord which caused 2 deaths This is attributed to insufficient intake of vitamin B_{12} combined with a high intake of folic acid

Dr J R Evans of London described absorption studies made with radioactive vitamin B_{12} In patients with pernicious anemia only 10 per cent of a dose of vitamin B_{12} is absorbed compared to a normal absorption of 50 to 75 per cent Absorption is increased if intrinsic factor is administered Patients who have had a total gastrectomy show diminished absorption because the source of intrinsic factor has been removed With partial gastrectomy however this area is retained Patients with idiopathic steatorrhea and intestinal infections also showed diminished absorption of vitamin B_{12} It is doubtful if the vitamin B_{12} formed in the intestines by bacterial action is available because if the intestinal wall is sterilized the amount of the vitamin absorbed is unchanged

285 Doscherholmen A and Hagen P S Radioactive vitamin B_{12} absorption studies results of direct measurement of radioactivity in the blood J Clin Investigation 35 699 June 1956 (in Soc Proc)

The absorption of vitamin B_{12} was determined by measurement of radioactivity of blood samples collected after the oral administration of a test dose of radioactive vitamin B_{12} Test doses consisted of 0 46 mcg and 0 92 mcg of vitamin B_{12} containing 0 5 microcurie and 1 0 microcurie of Co^{60} respectively Peak radioactivity in the blood samples did not appear until 8 to 12 hours after the test dose was given

Seventeen of 20 non pernicious anemia patients were given the 0 46 mcg dose In 6 tests whole blood net counts ranged from 18 to 41 per minute while in 14 instances plasma counts ranged from 19 to 54 In 3 given the larger test dose whole blood net counts ranged from 33 to 55 while plasma counts were 56 to 108 In 4 patients with pernicious anemia given the 0 46 mcg 0 5 microcurie dose the blood and plasma counts were not significant ranging from 2 to 6 per minute In one pernicious anemia patient given the dose containing 1 0 microcurie of Co^{60} net counts of 10 and 11 per minute were obtained in the whole blood and plasma respectively However with po-

tent intrinsic factor concentrate the blood or plasma radioactivity was within the range found in the non pernicious anemia patients

Minneapolis Minn

286 Pollycove, M and Apt L Absorption, elimination and excretion of orally administered vitamin B_{12} in normal subjects and in patients with pernicious anemia, New England J Med 255 207-212, Aug 2, 1956

Knowledge of the intestinal absorption of vitamin B_{12} is valuable in the diagnosis of addisonian pernicious anemia in patients with achlorhydria who are receiving maintenance therapy with liver or vitamin B_{12} Such absorption may be tested by measuring the hepatic uptake of orally administered Co^{60} vitamin B_{12}, or its excretion in urine or feces All three methods were used in 3 pernicious anemia patients and in 3 normal subjects with fairly consistent results The advantages of the three methods are discussed The urinary excretion test is most rapid but does not directly measure the amount of vitamin absorbed The liver scintillation count is the simplest easiest, and most accurate but requires at least seven days for completion The fecal excretion method takes at least 10 days but provides a direct measurement of the amount of vitamin B_{12} excreted

Tufts College Medical School and Boston Veterans Administration Hospital Boston Mass

287 Bunge, M B, Schloesser L L and Schilling R F Intrinsic factor studies IV Selective absorption and binding of cyanocobalamin by gastric juice in the presence of excess pseudovitamin B_{12} or 5 6-dimethylbenzimidazole J Lab & Clin Med 48 735 744, Nov 1956

The selective absorption and binding of vitamin B_{12} by gastric juice and serum in the presence of pseudovitamin B_{12} or 5 6-dimethylbenzimidazole (a moiety of the cyanocobalamin molecule) were studied *in vitro* and also in 6 patients who lacked intrinsic factor (pernicious anemia 4, subtotal gastrectomy 2)

Each patient received 1 000 mcg of crystalline vitamin B_{12} subcutaneously two hours after orally administered doses of normal human gastric juice and 1 mcg Co^{60} vitamin B_{12} plus 50 mcg vitamin B_{12} 50 mcg pseudovitamin B_{12} or 5 mcg 5 6-dimethylbenzimidazole Neither pseudovitamin B_{12} nor 5 6-dimethylbenzimidazole had any effect or showed competition with radioactive cyanocobalamin for mechanisms affecting vitamin B_{12} absorption

To test flushing action, 1 000 mcg doses of vitamin B_{12} and pseudovitamin B_{12} were administered subcutaneously two hours after administration of 1 mcg of oral Co^{60} vitamin B_{12} to 2 patients with pernicious anemia and to 4 control subjects The urine radioactivity was markedly less when pseudovitamin B_{12} was used although it had some flushing action

In vivo binding was studied in 2 control subjects Similar amounts of vitamin B_{12} or pseudovitamin B_{12} were excreted in the urine 24 hours after intramuscular injection of 122 mcg Administering excess vitamin B_{12}, pseudovitamin B_{12} or 5 6-dimethylbenzimidazole showed that the *in vitro* binding of cyanocobalamin by gastric juice was a selective process with a preference for vitamin B_{12} over pseudovitamin B_{12} and 5 6-dimethylbenzimidazole

The binding capacity of serum was not entirely selective Both vitamin B_{12} and pseudovitamin B_{12} reduced binding of Co^{60} vitamin B_{12} by serum 5 6-dimethylbenzimidazole however did not have this effect

The authors conclude The data are in accord with the hypothesis that binding of cyanocobalamin is necessary for intrinsic factor activity but binding alone

is no criterion for the presence of such activity

University of Wisconsin Medical School
Madison, Wis

Merck Sharp &
Dohme Research Laboratories
Rahway N J

288 Rath C. E. McCurdy P R and Duffy B J Jr Effect of renal disease on the Schilling test New England J Med 256 111 114 Jan 17 1957

The widespread use of panhematinics containing folic acid has made the diagnosis of pernicious anemia difficult The Schilling test utilizing Co^{60} vitamin B_{12} which has become a valuable adjunct in diagnosis, may be limited in renal disease The results of this test were studied in 33 control subjects in 29 patients with pernicious anemia and in 16 with severe renal disease Each subject (fasting) was given 1 000 mcg of nonradioactive vitamin B_{12} intravenously 30 minutes after the ingestion of 0 1 microcurie of Co^{60} vitamin B_{12} made up to a total of 0 5 mcg A few patients received daily intramuscular flushing doses of 1 000 mcg of nonradioactive vitamin B_{12}

The 24-hour urinary excretion in the 29 patients with Addison s pernicious anemia was less than 6 9 per cent of the oral dose The 33 normal subjects excreted 8 4 per cent or more the majority over 10 per cent Of the 16 patients with renal disease 13 excreted 5 7 per cent or less of the oral dose There was no correlation between urinary volume blood urea nitrogen and the urinary excretion of the radioactive vitamin Intrinsic factor administered to 2 patients did not increase the excretion of the vitamin This suggests that the defect is not an effect of uremia on intrinsic factor formation However there was a considerable excretion of radioactive vitamin B_{12} after the first 24 hours in the 7 patients with renal disease in contrast to the 3 patients with pernicious anemia These observations suggest that the low rates of excretion in some patients with renal disease are due to a delay in excretion resulting from impaired glomerular filtration in the presence of relatively intact tubular reabsorption This delayed excretion might be a useful aid in the differential diagnosis of pernicious anemia and renal disease in which routine urinalysis and clinical evaluation disclose little information

Georgetown University
School of M dicine and Hospital
Washington D C

289 Berlyne G M Liversedge, L A and Emery E W Radioactive vitamin B_{12} in the diagnosis of neurological disorders Lancet 1 294-296 Feb 9 1957

The Schilling test for vitamin B_{12} absorption was used diagnostically in 25 patients with neurologic disorders The patients were given 0 5 microcurie of Co^{60} vitamin B_{12} to which nonradioactive vitamin B_{12} was added to make a total of 3 mcg of vitamin B_{12} per cc This was given by mouth in 100 cc of water Two hours later 1 000 mcg of ordinary vitamin B_{12} was given intramuscularly to ensure high serum levels and maximum excretion Urine excreted during the 24 hours following ingestion of Co^{60} vitamin B_{12} was assayed for radioactivity

The test was first done on 5 normal subjects The excretion was 4 0 to 7 1 per cent a range lower than the 7 to 22 per cent reported by Schilling The lower values may have been due to age since none of the 5 subjects were under 45

In 10 patients with subacute combined degeneration the excretion range was 0 3 to 1 0 per cent When the test was repeated in 4 patients with the addition of 100 mg of intrinsic factor to the Co^{60} vitamin B_{12} the excretion rose to 4 1 to 6 0 per cent

In 15 patients with myeloneuropathies this test made it possible to rule out subacute combined degeneration, since excretion of vitamin B_{12} was normal.

tent intrinsic factor concentrate the blood or plasma radioactivity was within the range found in the non pernicious anemia patients

Minneapolis Minn

286 Pollycove, M and Apt, L Absorption, elimination and excretion of orally administered vitamin B_{12} in normal subjects and in patients with pernicious anemia, New England J Med 255 207-212 Aug 2, 1956

Knowledge of the intestinal absorption of vitamin B_{12} is valuable in the diagnosis of addisonian pernicious anemia in patients with achlorhydria who are receiving maintenance therapy with liver or vitamin B_{12} Such absorption may be tested by measuring the hepatic uptake of orally administered Co^{60} vitamin B_{12} or its excretion in urine or feces All three methods were used in 3 pernicious anemia patients and in 3 normal subjects with fairly consistent results The advantages of the three methods are discussed The urinary excretion test is most rapid but does not directly measure the amount of vitamin absorbed The liver scintillation count is the simplest easiest and most accurate but requires at least seven days for completion The fecal excretion method takes at least 10 days but provides a direct measurement of the amount of vitamin B_{12} excreted

Tufts College Medical School and Boston Veterans Administration Hospital Boston Mass

287 Bunge, M B, Schloesser L L and Schilling, R F Intrinsic factor studies IV Selective absorption and binding of cyanocobalamin by gastric juice in the presence of excess pseudovitamin B_{12} or 5 6-dimethylbenzimidazole J Lab & Clin Med 48 735-744, Nov 1956

The selective absorption and binding of vitamin B_{12} by gastric juice and serum in the presence of pseudovitamin B_{12} or 5 6-dimethylbenzimidazole (a moiety of the cyanocobalamin molecule) were studied *in vitro* and also in 6 patients who lacked intrinsic factor (pernicious anemia 4 subtotal gastrectomy, 2)

Each patient received 1,000 mcg of crystalline vitamin B_{12} subcutaneously two hours after orally administered doses of normal human gastric juice and 1 mcg Co^{60} vitamin B_{12} plus 50 mcg vitamin B_{12} 50 mcg pseudovitamin B_{12} or 5 mcg 5 6-dimethylbenzimidazole Neither pseudovitamin B_{12} nor 5 6-dimethylbenzimidazole had any effect or showed competition with radioactive cyanocobalamin for mechanisms affecting vitamin B_{12} absorption

To test flushing action 1 000 mcg doses of vitamin B_{12} and pseudovitamin B_{12} were administered subcutaneously two hours after administration of 1 mcg of oral Co^{60} vitamin B_{12} to 2 patients with pernicious anemia and to 4 control subjects The urine radioactivity was markedly less when pseudovitamin B_{12} was used, although it had some flushing action

In vivo binding was studied in 2 control subjects Similar amounts of vitamin B_{12} or pseudovitamin B_{12} were excreted in the urine 24 hours after intramuscular injection of 122 mcg Administering excess vitamin B_{12} pseudovitamin B_{12} or 5 6-dimethylbenzimidazole showed that the *in vitro* binding of cyanocobalamin by gastric juice was a selective process with a preference for vitamin B_{12} over pseudovitamin B_{12} and 5 6-dimethylbenzimidazole

The binding capacity of serum was not entirely selective Both vitamin B_{12} and pseudovitamin B_{12} reduced binding of Co^{60} vitamin B_{12} by serum 5,6-dimethylbenzimidazole however, did not have this effect

The authors conclude "The data are in accord with the hypothesis that binding of cyanocobalamin is necessary for intrinsic factor activity but binding alone

tients with renal insufficiency emphasiz ing the importance of collecting the urine beyond 24 hours Further evidence of de layed excretion was seen when 1 control and 1 patient with renal insufficiency were given 1 000 mcg of labeled vitamin B_{12} intravenously In the normal subject 96 per cent of the dose had left the plasma in four hours and 86 per cent was ex creted in the urine in 24 hours compared to 78 per cent and 47 per cent in the pa tient with renal insufficiency

Boston University School of Medicine and Tufts University School of Medicine Boston Mass

292 Goldberg S R Trivedi B K and Olner L. Radioactive vitamin B_{12} studies Experience with the urinary excretion test and the measurement of absorbed plasma radioactivity J Lab & Clin Med 49 583 589 April 1957

Vitamin B_{12} activity in urine and plas ma were compared in normal subjects and in patients with pernicious anemia The urinary test was done on 19 normal and 7 pernicious anemia patients and the plasma studies on 16 normal and 5 pernicious anemia patients The urinary excretion (modified Schilling test) was measured in the 24-hour urine collected after the patient received 0 46 mcg of Co^{60} vitamin B_{12} orally followed in one hour by a 100 mcg flushing dose of non radioactive vitamin B_{12} given intramus cularly Plasma radioactivity was meas ured after 1 mcg or more of either Co^{60} or Co^{58} vitamin B_{12} was given orally with out a flushing injection Smaller doses did not give detectable plasma levels

The 24-hour urinary excretion of Co^{60} vitamin B_{12} in healthy subjects ranged from 6 2 to 33 4 per cent (mean 17 9 per cent) of the dose while pernicious anemia patients excreted from 0 20 to 3 74 per cent Of 5 patients with renal disease 3 had excretion within the normal range but 2 with low urine volumes fell within the pernicious anemia range

Peak plasma radioactivity following 2 mcg or less of vitamin B_{12} occurred 10 hours after ingestion then declined but was still significant at 24 hours The range was 0 23 to 1 71 per cent (mean 0 8 per cent) of dose per liter of plasma for nor mal subjects and a maximum of 0 12 per cent per liter of plasma in patients with pernicious anemia Previous studies showed that following potent intrinsic factor preparations absorption curves of pernicious anemia patients were similar to those of normal subjects

Maximum plasma radioactivity fol lowing 4 mcg doses of vitamin B_{12} in the 3 normal subjects given this dose was about half the mean value of 0 8 per cent found at 10 hours after the smaller dose This apparently bore out previous reports that gastrointestinal absorption of vita min B_{12} is decreased if the amount given exceeds 1 5 to 2 0 mcg One of the 2 per nicious anemia patients given 4 mcg had plasma radioactivity levels within the normal range This is said to imply that with larger amounts of vitamin B_{12} there may be a process other than active ab sorption possibly simple diffusion

The authors state that the plasma ra dioactivity method is advantageous since it does not require the patient to collect a specimen

Michael Reese Hospital Chicago Ill

293 Doscherholmen A and Hagen P S Radioactive vitamin B_{12} absorption studies results of direct measure ment of radioactivity in the blood Blood 12 336-346 April 1957

A method of studying the absorption of oral vitamin B_{12} by direct measure ment of radioactivity of the blood was tested in 36 control subjects and in 9 pa tients with pernicious anemia Oral doses of 0 46 or 0 92 mcg (0 5 or 1 microcurie) of Co^{60} vitamin B_{12} were given in the fasting state and the radioactivity of the blood or plasma was measured at inter

A patient with subacute combined degeneration and diabetes excreted only 0 3 per cent of the ingested radioactive vitamin B_{12} When the test was repeated with the addition of 100 mg of intrinsic factor excretion rose to 4 1 per cent With vitamin B_{12} therapy, neurologic symptoms cleared in three months

University of Manchester and Manchester Royal Infirmary Manchester England

290 Citrin Y, DeRosa, C and Halsted, J A Locus of absorption of vitamin B_{12} Federation Proc 16 353, March 1957

In subjects with and without pernicious anemia physiologic doses (0 5 5μg) $Co^{60}B_{12}$ was delivered by intestinal intubation to various levels of the intestinal tract Using 2 flushing doses absorption was assessed by the urinary excretion test, urine being collected for 48 hours Good reproductibility in the same individual was demonstrated When $Co^{60}B_{12}$ was delivered to the duodenum of 5 normal subjects from 15 6-39 5% was recovered In 4 recovery following duodenal instillation was significantly greater than after oral administration After jejunal instillation 12 3 22.3 and 22 8% recovery, respectively occurred in 3 subjects In 6 subjects the test dose delivered to the lower ileum resulted in urinary recoveries of 0 0, 4 7 16 5 18 4 and 23 2% respectively In 4 patients with pernicious anemia the test dose to which was added a potent intrinsic factor preparation was delivered to the lower ileum Significant absorption occurred in all Without added intrinsic factor no absorption was demonstrable Fourteen test doses alone with intrinsic factor or after Neomycin sterilization instilled into the sigmoid of normal subjects and 1 dose with intrinsic factor instilled into the transverse colon resulted in no absorption These results suggest that the lower small intestine but not the large bowel, is capable of absorption of B_{12} in physiologic amounts and that absorption from the ileum requires the presence of intrinsic factor '

State University of New York College of Medicine Syracuse N Y

291 Miller, A, Corbus H F and Sullivan, J F A modified urinary excretion test for measuring oral cobalt[60] labeled vitamin B_{12} absorption and its application in certain disease states, Blood 12 347-354, April 1957

Factors influencing the results of the urinary excretion test for oral vitamin B_{12} include the time of the first flushing dose the effect of the second flushing dose, and the size of the oral dose A modified test is presented in which an oral dose of 0 48 mcg of Co^{60} vitamin B_{12} is given to the fasting patient followed by two flushing doses of 1 mg of vitamin B_{12} given intramuscularly 3 and 24 hours later Urine is collected for two 24-hour periods and the radioactivity is measured

Results of the Modified Test

Disease	Patients	Urinary excretion (% of dose)
Controls	16	21 to 48%
Pernicious Anemia	9	1 to 7%
Combined System Disease without anemia, receiving vitamin B	3	2 to 15%
Bacterial Infection		
Acute	3	1 to 9%
Convalescent	5	18 to 47%
Renal Disease	3	27 to 51%
Terminal	1	0 to 3%
Miscellaneous Diseases	16	17 to 50%

The modified test produces counting rates in normals which are far greater than those in patients with pernicious anemia thus increasing diagnostic accuracy In other disease states only patients with sprue and a patient with exfoliative dermatitis receiving cortisone had values clearly below the normal range

Results in patients with acute infection and severe uremia appear invalid A delay in urinary excretion occurred in pa

termine the vitamin B_{12} activity in the urine after nasal application

Normal subjects and pernicious anemia patients excrete vitamin B_{12} in the urine in similar quantities following parenteral injection The urinary vitamin B_{12} activity following intranasal instillation of the vitamin suggests similar patterns but smaller quantities are excreted than after parenteral administration of equal amounts A pernicious anemia patient in relapse showed a reticulocytosis of 44 3 per cent when 150 mcg of crystalline vitamin B_{12} was applied directly to the mucous membrane of the nasal turbinate bone A single attempt to demonstrate intranasal binding of vitamin B_{12} with nasal mucus was unsuccessful Crystalline vitamin B_{12} in saline solution and as crystals is rapidly absorbed and excreted by the kidneys when applied to the nasal mucosa

Henry Ford Hospital
Detroit Mich

296 Monto R. W and Howell J T Urinary vitamin B_{12} activity following intranasal administration J Lab & Clin. Med 45 474-477, March 1955

On separate days nasal instillations of 200 100 and 50 mcg of crystalline vitamin B_{12} in 0 5 cc saline were given to 2 normal subjects In addition 2 patients with pernicious anemia in relapse received under direct observation, upon the nasal mucosa 200 mcg and 150 mcg respectively of vitamin B_{12} crystals Urine specimens were collected for a two-hour period before administration and at two five, and eight hours afterward Assays for vitamin B_{12} activity utilized *L. leichmannii*

In the test period significant amounts of urinary vitamin B_{12} activity were detected which were proportional to the amounts given orally The urinary excretion patterns were similar to those obtained by parenteral injection Apparently the vitamin is absorbed directly by the nasal mucosa

Crystalline vitamin B_{12} crystals produced no irritation of the nasal mucous membranes In 1 patient numerous nasal polyps and hypertrophy of the mucous membranes apparently did not impede absorption of the vitamin

Henry Ford Hospital
Detroit Mich

MICROBIOLOGICAL ASSAYS

297 Gregory M E. The microbiological assay of vitamin B_{12} in the milk of different animal species Brit J Nutrition 8 340-347, 1954

Colostrum of cow and goat and the milk of cow goat, sheep pig and rat as well as of humans were assayed for vitamin B_{12} by three different organisms with the following results

Mean Values From 2 Assays

Species	L. leichmannii assay (mμg /ml)	Esch coli assay (mμg /ml)	Ochromonas malhamensis assay (mμg /ml)
		Milk	
Cow	2 3	1 8	2 3
Goat	0 9	1 1	0 8
Woman	0 2	0 2	0 2
Sow	0 0	0 0	0 0
Rat	9 6	8 9	8 7
Ewe	10 0	8 2	10 3
		Colostrum	
Cow	3 9	3.2	4 0
Goat	7 1	6.2	6 3

Various methods for preparing the milk samples for assay were compared The vitamin B_{12} activity of the milks was due *almost entirely to cyanocobalamin In all* milk tested the cyanocobalamin was present in bound form

298 Haenel H The evaluation of the Euglena test (assay of vitamin B_{12}) with the aid of ultra sound Naturwiss 41 143-144 1954

The evaluation of the Euglena test by means of turbidimetry was not feasible because the organisms are too large (50

vals up to one week Plasma gave slightly higher counts

In control patients given the smaller dose, measurable activity appeared in most at 4 hours reached a peak at 8 to 12 hours and thereafter slowly declined with significant activity persisting even after a week This absorption curve differed from the early rise observed by others after massive oral doses of vitamin B_{12}, indicating a different mode of absorption It also differed from the rapid disappearance after parenteral administration previously reported The patients with pernicious anemia showed no radioactivity, or only traces, unless intrinsic factor concentrate was given, counts were then within normal range Results were similar when the larger dose was used except that the counts were higher

The above method was compared with a modified Schilling test in 11 control subjects, and in 7 patients with pernicious anemia with and without the use of intrinsic factor In the Schilling test, 1 000 mcg of vitamin B_{12} was given intramuscularly 24 hours after the oral test dose of 0 46 mcg of Co^{60} vitamin B_{12} A correlation was found between urinary radioactivity and the plasma or blood activity Duplicate tests on 5 control subjects revealed good reproducibility in the tests, the repeated plasma values correlated a little better than the duplicate urinary excretion percentages

University of Minnesota Medical School and Veterans Administ ation Hospital Minneapolis Minn

294 Rosenblum, C Davis, R L and Chow, B F Comparative absorption of vitamin B_{12} analogues by normal humans III 5,6-Dichlorobenzimidazole, 5 6-desdimethylbenzimidazole and 5 hydroxybenzimidazole analogues, Proc Soc Exper Biol & Med 95 30-32, May 1957

The absorption of vitamin B_{12} analogues was studied in normal human subjects Doses of 1 1 4 and 2 mcg of labeled, 5,6-dichlorobenzimidazole analogue (DCBA), 5 6-desdimethylbenzimidazole analogue (DMBA) and 5 hydroxy benzimidazole analogue (Bernhauer s Factor III) were given Two hours later 1 mg of unlabeled derivative was injected and urinary excretion was measured at 24 hours and in some cases at 48 and 72 hours after daily flushing doses

Slow elimination was not a factor, as one and three-day excretion values did not differ greatly None of the analogues were absorbed to the extent that cyanocobalamin is Desdimethyl and hydroxy benzimidazole were absorbed to only a slight extent the latter being absorbed the least The absorption of the dichlorobenzimidazole analogue was approximately one half that of cyanocobalamin

Clearly, factors other than the cyano group affect oral absorption, since all three analogues contain cyanide complexes Of these DCBA with its 2 symmetrically distributed chlorine atoms evinces the greatest absorption tendency One is, accordingly tempted to suggest that the 5,6 disubstitution is a favorable configuration, and that the difference between DCBA and cyanocobalamin lies in preference of the human organism for methyl rather than chloro groups "

Merck Sharp & Dohme Research Laboratories Rahway N J

Johns Hopkins University and Veterans Administration Hospital Baltimore Md

295 Monto, R. W and Howell J T Absorption and excretion of crystalline vitamin B_{12} when applied to the nasal mucosa Clin Research Proc. 2 55 56 April 1954

Recent reports on inhalation or nasal instillation of vitamin B_{12} for pernicious anemia suggest that contact with intrinsic factor is not a prerequisite for the absorption of vitamin B_{12} through the respiratory mucous membrane In view of these observations it is pertinent to de

termine the vitamin B_{12} activity in the urine after nasal application

Normal subjects and pernicious anemia patients excrete vitamin B_{12} in the urine in similar quantities following parenteral injection The urinary vitamin B_{12} activity following intranasal instillation of the vitamin suggests similar patterns but smaller quantities are excreted than after parenteral administration of equal amounts A pernicious anemia patient in relapse showed a reticulocytosis of 44 3 per cent when 150 mcg of crystalline vitamin B_{12} was applied directly to the mucous membrane of the nasal turbinate bone A single attempt to demonstrate intranasal binding of vitamin B_{12} with nasal mucus was unsuccessful Crystalline vitamin B_{12} in saline solution and as crystals is rapidly absorbed and excreted by the kidneys when applied to the nasal mucosa

Henry Ford Hospital
Detroit Mich

296 Monto R. W and Howell J T Urinary vitamin B_{12} activity following intranasal administration J Lab & Clin. Med 45 474-477, March 1955

On separate days nasal instillations of 200 100 and 50 mcg of crystalline vitamin B_{12} in 0 5 cc saline were given to 2 normal subjects In addition 2 patients with pernicious anemia in relapse received under direct observation upon the nasal mucosa 200 mcg and 150 mcg respectively of vitamin B_{12} crystals Urine specimens were collected for a two-hour period before administration and at two five and eight hours afterward Assays for vitamin B_{12} activity utilized *L leichmannii*

In the test period significant amounts of urinary vitamin B_{12} activity were detected which were proportional to the amounts given orally The urinary excretion patterns were similar to those obtained by parenteral injection Apparently the vitamin is absorbed directly by the nasal mucosa

Crystalline vitamin B_{12} crystals produced no irritation of the nasal mucous membranes In 1 patient numerous nasal polyps and hypertrophy of the mucous membranes apparently did not impede absorption of the vitamin

Henry Ford Hospital
Detroit Mich

MICROBIOLOGICAL ASSAYS

297 Gregory, M E The microbiological assay of vitamin B_{12} in the milk of different animal species Brit J Nutrition 8 340-347, 1954

Colostrum of cow and goat and the milk of cow goat sheep pig and rat, as well as of humans were assayed for vitamin B_{12} by three different organisms with the following results

Mean Values From 2 Assays

Species	L. leichmannii assay (mμg /ml)	Esch coli assay (mμg /ml)	Ochromonas malhamensis assay (mμg /ml.)
		Milk	
Cow	2 9	1 8	2.3
Goat	0 9	1 1	0 8
Woman	0 2	0.2	0.2
Sow	0 0	0 0	0 0
Rat	9 6	8 9	8 7
Ewe	10 0	8 2	10 3
		Colostrum	
Cow	3 9	3.2	4.0
Goat	7 1	6 2	6.3

Various methods for preparing the milk samples for assay were compared The vitamin B_{12} activity of the milks was due almost entirely to cyanocobalamin In all milk tested the cyanocobalamin was present in bound form

298 Haenel H The evaluation of the Euglena test (assay of vitamin B_{12}) with the aid of ultra sound Naturwiss 41 143 144 1954

The evaluation of the Euglena test by means of turbidimetry was not feasible because the organisms are too large (50

to 60 microns) for this technic When exposed to ultra sound for one minute the organisms are broken down into particles averaging 3 to 6 microns which brings them into the range of turbidimetric measurements

Anstalt für Vitaminforschung
Potsdam Rehbrücke Germany

299 **Wolff, R and Dubost, S Fixation of vitamin B_{12} by Lactobacillus leichmannii and its application to the determination of bound vitamin B_{12} in organ extracts Biochim Biophys Acta 14 576-577, Aug 1954**

Bound vitamin B_{12} may be determined in a mixture containing both the bound and free form treatment with *L leichmannii* eliminates the free vitamin

University of Nancy
Nancy France

300 **Bandelin, F J and Tuschhoff, J V The microbiological determination of vitamin B_{12} utilizing a mutant strain of Escherichia coli, J Am Pharm A 43 474-477, Aug 1954**

A turbidimetric method for the assay of vitamin B_{12} utilizing a mutant strain of *Escherichia coli* is described The growth response curve is reproducible within satisfactory limits Methionine interferes only when present in amounts 70 000 times that of the vitamin B_{12} The recovery of cyanocobalamin added to various liver extracts ranges from 96 to 106 per cent Determinations carried out on a number of injectable liver and desiccated liver extracts indicate that the *E coli* mutant method gives results which are comparable to those obtained with the official *Lactobacillus leichmannii* method

It is stated that when methionine interference can be avoided this turbidimetric method may have considerable value because of its simplicity and speed

Flint Eaton & Company
Decatur Ill

301 **Grossowicz N, Aronovitch, J and Rachmilewitz, M Determination of vitamin B_{12} in human serum by a mutant of Escherichia coli, Proc Soc Exper Biol & Med 87 513 514, Dec 1954**

The vitamin B_{12} in human serum was assayed by use of a mutant of *Esch coli*

The assay method is accurate and simple requiring only 40 to 48 hours The vitamin B_{12} content of sera of healthy subjects ranged from 200 to 1,000 mcg per cc, in pernicious anemia patients it ranged from 50 to 130 mcg per cc

Hebrew University
Hadassah Medical School
Jerusalem Israel

302 **Ostergaard Kristensen, H P Investigations into the Euglena gracilis method for quantitative assay of vitamin B_{12} Acta physiol Scandinav 33 232 237, 1955**

Author s summary Using Ross s modification of Hutner s method for quantitative determination of vitamin B_{12} with *Euglena gracilis* var *bacillaris* experiments were carried out which showed that the supernatant from a centrifuged vigorously growing Euglena culture contains a thermolabile factor which has a growth inhibiting effect on Euglena cells when they are transferred to a freshly prepared medium containing vitamin B_{12}

Density and uniformity of growth in the tubes inoculated are increased by washing and diluting the Euglena culture used for inoculation The sensitivity and the accuracy of the method are thereby increased

University of Copenhagen
Copenhagen Denmark

303 **Biological and microbiological methods of estimating vitamin B_{12} Nature 176 384-385, Aug 27, 1955**

Summaries of papers were read at a conference on assay of vitamin B_{12} It was stated that the *L lactis* assay has largely

been superseded by improved micro biological techniques which employ several organisms Complicating factors in such assays include the presence of materials closely related to vitamin B_{12}

Assay with *L leichmannii* was described and it was stated that probably the best method of measuring serum vitamin B_{12} levels is to use both this organism and *Euglena gracilis* Euglena provides a highly sensitive method which in serum is specific for vitamin B_{12}

The problems of using *L. leichmannii* and *Ochromonas malhamensis* for assay of vitamin B_{12} in animal foodstuffs were also discussed The *L leichmannii* assay more closely approximates results by chick assay

In assay of vitamin B_{12} in milk *Ochromonas malhamensis Esch coli* and *L leichmannii* give closely similar results but the latter is preferred because it provides a simpler method

304 Berman D Yacowitz H and Weiser H H A differential microbiological assay for vitamin B_{12} and pseudovitamin B_{12} Appl Microbiol 4 49 52, Jan 1956

The method employs a differential assay using *L. leichmannii* 7830 and *L acidophilus* 832 as the test organisms

Ohio State University
Columbus Ohio

305 Williams W L Stiffey V and Jukes T H Microbiological and chick assay of vitamin B_{12} activity in feed supplements and other natural products Agr & Food Chem 4 364-367, April 1956

Recent modifications and improvements of the *Ochromonas* and *Esch coli* methods as well as the details of a chick growth method for determining vitamin B_{12} are presented Certain fermentation materials contain substances which inhibit chick growth so that the chick assay method cannot be used in such cases

American Cyanamid Company
Pearl River N Y

306 Stapert E M, Goff J W and Stubberfield, L A vitamin B_{12} binding substance obtained from L leichmannii 7830, J Am Pharm A (Sci ent Ed) 45 309 311 May 1956

A substance which binds vitamin B_{12} was obtained from the *L leichmannii* 7830 organism and the U S P medium in which this organism was cultured This substance inhibits the growth of *L leichmannii* 7830

The Upjohn Company
Kalamazoo Mich

307 Robinson F A Fitzgerald M E H and Grimshaw J J The microbiological assay of vitamin B_{12} in liver extracts I Experimental design and problems of validity J Pharm Pharmacol 8 635-647, Sept 1956

Experimental methods are given for the assay of cobalamins in liver extracts using plate and tube assay methods with *B coli* N C T C 8134 and *L leichmannii* N C T D 7854 as test organisms

Allen & Hanburys Ltd
London England

GENERAL

VITAMIN B_{12} CONTENT, ACTIVITY

308 *Rosenthal H L* and Brown C L, Jr Vitamin B_{12} activity of plasma and whole blood from various animals Proc Soc Exper Biol & Med. 86 117 120 May 1956

Authors summary Vitamin B_{12} activity of whole blood and plasma from the human dog calf rabbit, chicken and alligator was studied before and after

alkaline hydrolysis The data show a wide variation of blood and plasma vitamin B_{12} content between the different species In mammals, the vitamin is almost equally distributed between the erythrocytes and plasma In chicken and alligator, the major portion of the vitamin activity is associated with the nucleated erythrocyte

Tulane University School of Medicine
New Orleans La

309 Robinson, F A, Fitzgerald, M E H, Fehr, K and Grimshaw, J J Vitamin B_{12} in crude liver extracts, Nature 174 558-559, Sept 18, 1954

Crude liver extracts contain a vitamin B_{12} binding substance and the vitamin B_{12} is partly present in bound form, however, binding is not due to the formation of a complex with a protein The conclusion of other investigators that in liver concentrates, cyanocobalamin usually accounts for only a small fraction of total cobalamins is confirmed

Allen & Hanburys Ltd
London England

310, Hartman A M, Dryden, L P and Riedel G H Vitamin B_{12} content of milk and milk products as determined by rat assay, J Nutrition 59 77-88, May 1956

A vitamin B_{12} assay method using growth of the depleted normal rat as the criterion of potency has been described and utilized to assay milk and milk products The average vitamin B_{12} content of 10 lots of raw whole milk was found to be 7 1 mcg per liter Neither pasteurization of milk nor its storage at 0 C for three days had any effect on its vitamin B_{12} potency

U S Department of Agriculture
Beltsville Md

STABILITY STUDIES

311 Bartilucci, A and Foss, N E Cyanocobalamin (vitamin B_{12}) I A study of the stability of cyanocobalamin and ascorbic acid in liquid formulations, J Am Pharm A (Scient Ed) 43 159 162, March 1954

Authors summary 'The optimum pH for stability of ascorbic acid in solution was found to be above 6 0 but below 7 0 The optimum pH for cyanocobalamin is between 4 5 and 5 0 The most favorable pH for a mixture of cyanocobalamin and ascorbic acid appears to be between 6 0 and 7 0

' Ascorbic acid was found to be most stable in high concentrations of propylene glycol with distilled water glycerin or Sorbo and a mixture of glycerin and Sorbo Cyanocobalamin was found to be stable in a wide variety of vehicles It has questionable stability in high concentrations of propylene glycol and in vehicles containing glucose A vehicle composed of equal parts of propylene glycol and glycerin afforded maximum stability conditions for a mixture of cyanocobalamin and ascorbic acid There was about 80 per cent retention of cyanocobalamin and 85 per cent retention of ascorbic acid in this vehicle after six months storage at 40 degrees There was no loss of cyanocobalamin and about 10 per cent loss of ascorbic acid on storage for six months at room temperature

' It is suggested that the decomposition products of ascorbic acid play an important part in the decomposition of cyanocobalamin Investigations are continuing on various phases of this problem, and it is expected that the results will be published

University of Maryland
School of Pharmacy
Baltimore Md

312 Blitz M, Eigen, E and Gunsberg E Vitamin B_{12} studies—the instability of vitamin B_{12} in the presence of thiamine and niacinamide J Am Pharm A (Scient Ed) 43 651-653 Nov 1954

Authors summary Vitamin B_{12} is not stable in a B-complex solution at pH

4.25 containing thiamine and niacinamide among its constituents

Oxidation appears not to be a factor in the decomposition of vitamin B_{12} in the B-complex solution

Large losses of vitamin B_{12} were found to be a function of the concentration of both thiamine and niacinamide in the solution

313 Shenoy, K. G and Ramasarma G B Iron as a stabilizer of vitamin B_{12} activity in liver extracts and the nature of so-called alkali-stable factor Arch Biochem & Biophys 55 293 295 March 1955

The fact that vitamin B_{12} is more thermostable in Liver Concentrate N F IX than in Liver Fraction 1 N F IX is due to the iron content of the former The vitamin B_{12} content of Liver Fraction 1 N F IX is destroyed by heating but added vitamin B_{12} can be protected by addition of $FeCl_3$

Raptakos Brett & Co
Bombay India

314 Macek, T J and Feller, B A A note on stability of the B vitamins in solutions—crystalline vitamin B_{12} J Am Pharm A. (Scient Ed) 44 254 April 1955

It is possible to prepare aqueous solutions of the B vitamins in which crystalline vitamin B_{12} is stable for prolonged periods of time at normal storage temperatures

Merck & Co Inc
Rahway N J

315 Feller B A and Macek T J Effect of thiamine hydrochloride on the stability of solutions of crystalline vitamin B_{12} J Am Pharm A (Scient Ed) 44 662-665 Nov 1955

Authors summary At room temperature crystalline vitamin B_{12} was found to be stable in aqueous solutions at pH 4 containing 10 mg per cc of thiamine hydrochloride alone or in combination with 10 mg per cc of niacinamide Decomposition of crystalline vitamin B_{12} in aqueous solution was increased appreciably in the presence of thiamine or thiamine decomposition products at elevated temperatures At elevated temperatures the thiazole moiety of the thiamine molecule decomposes crystalline vitamin B_{12} in aqueous solutions adjusted to pH 4 Data are given to show that crystalline vitamin B_{12} is stable in B vitamin solutions at pH 3 0 and 4 5 for long periods of time at normal storage temperatures but not when these are heated at 120 degrees

Merck & Co Inc
Rahway N J

316 DeMerre L J and Wilson C Photolysis of vitamin B_{12} J Am Pharm A (Scient Ed) 45 129 134 March 1956

Losses of vitamin B_{12} caused by exposure to various light sources are as follows sunlight (8 000 foot-candles) 10 per cent per half hour artificial light (14 000 foot-candles) about 12 per cent per half hour Effect of ultraviolet light is similar to that of sunlight with monochromatic light photolysis is higher in the short wave range Red light causes no destruction

Medical Laboratories
Army Chemical Center Md

317 Garrett, E R. Prediction of stability in pharmaceutical preparations II Vitamin stability in liquid multivitamin preparations J Am Pharm A (Scient Ed) 45 171 178 March 1956

The rates of thermal degradation of ascorbic acid vitamin B_{12} folic acid vitamin A d pentothenyl alcohol, and thiamine hydrochloride in a liquid multivitamin preparation have been studied at elevated temperatures and the orders of degradation determined Stability of vitamin components at room temperature was predicted by work performed in a relatively brief time and predictions con

firmed within the estimated error by room temperature shelf stability studies

The Upjohn Company
Kalama oo Mich

318 Garrett, E R Prediction of stability in pharmaceutical preparations III Comparison of vitamin stabilities in different multivitamin preparations, J Am Pharm A (Scient Ed) 45 470-473, July 1956

The rates of thermal degradation at elevated temperatures and heats of activation of vitamin B_{12} d pantothenyl alcohol and thiamine hydrochloride in a new liquid vitamin preparation have been determined Proof of the validity of a predictive method for room temperature stability is given and possible limitations are discussed

The Upjohn Company
Kalamazoo Mich

319 Hutchins H H Cravioto, P J and Macek, T J A comparison of the stability of cyanocobalamin and its analogs in ascorbate solution, J Am Pharm A (Scient Ed) 45 806-808, Dec 1956

Synopsis The stability of eight vitamin B_{12} products in 1 per cent ascorbic acid solution in 1 molar acetate buffer at pH 4 0 has been studied during storage at constant temperatures Cyanocobalamin (crystalline vitamin B_{12}) was shown to be markedly more stable than cyanide free B_{12} analogs in ascorbate solutions Vitamin B_{12} concentrates containing mixtures of cyanocobalamin and noncyano analogs were found to be less stable in ascorbate solution than concentrates containing cyanocobalamin exclusively The stability of vitamin B_{12} concentrates thus appears to be related directly to the concentration of cyanocobalamin present in the concentrate

Merck Sharp &
Dohme Research Laboratories
Rahway N J

320 Blitz M, Eigen, E and Gunsberg, E Studies relating to the stability of vitamin B_{12} in B-complex injectable solutions J Am Pharm A (Scient Ed) 45 803 806 Dec 1956

Synopsis Room temperature storage studies of various commercial B complex injectables demonstrate the instability of vitamin B_{12} in these products Experimental data are presented to show that vitamin B_{12} is unstable in the presence of thiamine and niacinamide at concentrations from 25 mg to 100 mg per cc of each component in solutions at pH 4 25 but is relatively stable at lower concentrations The mechanism of this decomposition is discussed

INDEX OF SUBJECTS

B

C

F

G

H

O

P

T

U

INDEX OF AUTHORS

D

E

F

Abstract

G

H

M VI 11501